The Micro4Biogas – Roadmap

Micro4Biogas is a new European research project that aims to develop a deeper understanding of anaerobic microbiomes in particular for biogas production. Here, new ways of manipulating anaerobic microbiomes are to be explored, especially with regard to bioaugmentation. The project includes a comprehensive analysis of the European biogas landscape followed by a policy analysis, which is to be published as an e-book in the framework of Micro4Biogas.

Disclaimer: The present work is still a draft version. It needs rephrasing, proof reading and some chapters are still under elaboration. Chapter 7 and 10 will be written in the future.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

MICRO4BIOGAS - ABSTRACT

Since the 30.06.2021, the European Commission is funding the Micro4Biogas research project. In the frame of the H2020 research programme, 14 partners from 6 European Countries will receive €5.7M for a duration of 4 years. The main goal of the project is to assess the manipulability of anaerobic microbiomes based on bioaugmentation. Before entering the anaerobic microverse, the present roadmap gives a detailed overview on the basic functionalities of anaerobic digester (AD) plants and as well the economic situation. This includes the technical functionality, different types of digester plants, but also inputs and outputs, such as substrates, biogas, emissions and digestate.

To reflect the European situation in the biogas sector, Micro4Biogas is focussing on the 6 countries, which are represented within the project: The situation of the respective countries is very different. Among the countries considered, Greece had the smallest number of biogas plants in 2020 (60 units). Outgoing from about 20 plants in 2011, more and more plants for the utilisation of agricultural residues have been built. Most of the Greek AD plants are now using substrates from agriculture. Finland has a similar situation to Greece. Spain and the Netherlands each had between 200 and 300 biogas plants in 2020. However, these are mostly landfills and digesters for sewage sludge. Belgium had a similar amount as Spain and the Netherlands in 2020 (approx. 200 plants), but about 50% received their substrates from agriculture. Germany plays a pioneering role in the European biogas landscape with currently 11,000 plants, which provide now more than 70,000 GWhs. More than 90% of these plants use agricultural residues. The rapid increase in the amount of AD installations was possible mainly due to legislative support. In Germany, the Renewable Energy Sources Act (EEG) established a feed-in tariff and grandfathering for biogas plants, which ensured a stable economic situation in the long term. It must be emphasised at this point that Germany started early with the construction of biogas plants. There were already more than 8,000 biogas plants in 2011. The case in Germany shows in a positive way what potential biogenic residues have. Supported by current political events (tensions with Russia and the war in Ukraine), this leads to continuously increasing prices per kWh, which in turn increases the competitiveness of biogas plants. This shows even more that biogas is a good alternative to fossil fuels. It also reduces the dependency of international fossil fuel trading. However, biogas plants are still dependent on economic support (e.g., through the feed-in tariff). Further developments are necessary to ensure that biogas plants have a long-term future. Here, new marketing strategies could take effect. Potential improvements could be achieved in relation to emissions trading, new kinds of substrates, or new ways to better integrate biogas plants into the bioeconomy (e.g., through hydrogen production or extraction of organic acids for fine chemistry). Another solution, which is the focus of Micro4Biogas, is the optimisation of biogas plants based on bioaugmentation and microbiome manipulation.

Bioaugmentation refers to the introduction of microorganisms into certain systems, such as contaminated soil, polluted water or even biogas plants. The challenge here, however, is to find microorganisms that could not only optimise the biogas process. These respective organisms must also be able to assert themself against the large number of existing, indigenous microorganisms.

To find microorganisms that are both efficient and robust, the Micro4Biogas project is specifically looking for microorganisms that are already native to biogas plants. Currently, the

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project partners are working on one of the most comprehensive metagenome studies in relation to biogas. About 80 different samples were collected from many biogas plants. Process chemical parameters were collected for the corresponding plants. Further analyses are now being carried out using 16S-rRNA gene amplicon high-throughput sequencing or metagenome sequencing. Numerous microorganisms are to be isolated from the most promising samples and tested for suitability for bioaugmentation. In line with the goal of optimising anaerobic microbiomes, the underlying roadmap also describes the structure of anaerobic microbiomes and addresses their manipulability. The word microbiome stands for the totality of all microorganisms found in a defined habitat. The microbiome in biogas plants comprises about 300 key microbial groups and is thus a highly diverse habitat. Besides bacteria, archaea, fungi, protista and bacteriophages are found. While the work in Micro4Biogas focuses on bacteria and archaea, the other microbial groups are also of interest for future projects. Both anaerobic fungi and bacteriophages have important functions in the anaerobic microbiome. Anaerobic fungi have outstanding degradation strategies and bacteriophages have considerable influence on the taxonomic composition. Nevertheless, the number of publications on these organisms in relation to biogas is extremely low. Amongst bacteria, the so-called Candida Phyla Radiation (CRP) could also play an importance in future research. One also speaks here of nanobacteria, and an interesting representative is *Gracilibacteria*. This genus is not viable on its own. They can physically dock to other microorganisms and are thus kept alive. They seem to have important functions in the degradation of substances that are difficult to break down, e.g. in petroleum-contaminated waters, and they have also been observed be present in taxonomic profiles, which comprise methanogenic archaea. The anaerobic treasure chest of the biogas sector certainly holds further surprises and Micro4Biogas would like to help realise this potential.

Independent of optimisation of anaerobic microbiomes, Micro4Biogas would also like to give an update on the current situation of the European biogas landscape. In this context, an intensive cooperation with the European Biogas Association (EBA) is planned and an extensive survey is already in preparation. At least 200 people are to be interviewed, whereby stakeholders but also people without a connection to biogas are eligible. Of interest is the general level of knowledge about the biogas industry, the future potential of biogas plants for the bioeconomy, technical and legal approaches to improving biogas plants, and the need for new funding instruments. The keyword "microbiome" also plays an important role here.

Finally, the cooperation with the town of Arras de los Olmos should be mentioned. The town is in the northernmost part of the Community of Valencia. Since the "Covenant of Mayors for Climate and Energy" in 2014, the town has committed itself to decarbonisation, capacity building, sustainable and affordable energy. The goal is a complete switch to renewable energy, and biogas will play a role here. Even though the goals of Arras de los Olmos are pursued independently of the Micro4Biogas project, the project consortium accompanies Arras de los Olmos on its way and presents their project as a case study in this roadmap. Arras de los Olmos is currently building a biogas plant. Should this be completed within the project framework, synergies with the Micro4Biogas project might be possible. The plant biogas plant might be available for test trials within the framework of Micro4Biogas.

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Specific tasks within Micro4Biogas

Task 1.1: State of the art analysis: Biogas production is an industrial area with various structural solutions. There are different types of biogas reactors, but several reactors of different designs can also be combined with one another. In addition, there are additional process differences with regard to the selected process chemical environmental parameters. In particular, the number of possible substrates is highly variable. Finally, many industrial areas are known that are related to the biogas industry. The first chapter on the project roadmap is intended to show the current status in the biogas industry and to reflect its versatility. We would like to present the structural standards of different types of biogas plants against the background of biological processes, operational safety, environmental friendliness, and ecological considerations. We would also like to point out weaknesses and opportunities for improvement.

Task 1.2: Policy Analysis: A comprehensive overview of the political situation, political objectives, target markets and future development opportunities for the biogas industry is planned. The role of biogas plants as a networking element of the bioeconomy and industry should be emphasized. In connection with MICRO4BIOGAS, three fundamental questions arise:

1. Can bioaugmentation improve the efficiency of biogas plants and thus the market situation for biogas plants?
2. Can multi-stage biogas plants help to substitute petroleum-based raw materials at least partially?
3. Could bioaugmentation help to better interconnect the biogas industry with other branches of industry in the biorefinery or with the provision of biological resources?

All three questions will be answered based on an extensive literature research. A survey with key players in the European biogas landscape is also to be carried out.

Biogas production, as commented above, is linked to agriculture and cattle industry among other sectors, having thus a great influence over the development of several EU rural areas. Micro4biogas will study which existing EU policies can foster biogas production in rural areas and how to improve them, as well as the existing rural networks able to push towards Biogas production. In addition, and linked to WP6, we will analyse the public funding options and alternatives in such a low margin market.

T1.3: New technologies and innovations: The rise of next-generation sequencing technologies in the last few years has stimulated the research of anaerobic digester microbiomes. This tremendous sequencing effort will now be gathered and exploited by the MICRO4BIOGAS consortium. With the help of curated literature and text mining strategies, all publicly available sequencing data and their associated scientific papers will be identified, integrated, and analysed, with a special focus on the manipulability of anaerobic microbiomes and on the accessibility and standardisation of the sequencing data, metadata, methods and protocols from each study. Once all possible data is integrated, several aspects and their effects

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on the anaerobic digestion process will be deeply analysed, for example, increase of hydrogen forming and synthrophic bacteria, DIET and its links with phototrophy, variation of typical process parameters (pH, Temperature, viscosity, etc.), increase of cellulase-producing bacteria, and alterations of signalling/quorum sensing. The final goals are to gain insight on potential bioaugmentation strategies, and to apply machine learning techniques to predict the behaviour of a specific microbiome and its implication in the biogas production process.

T1.4: AD-Microbiomes vs. Europe: The key contribution of this task will be the addition of industrial microbiome approach to the European biogas landscape (EBL). To do so, the EBL will be deeply characterized from several points of view:

1. innovations, technological advances and in silico analysis associated to this field;
2. public policies and public networks involving local governments; and
3. biogas stakeholders' map.

We will reinforce the biogas innovation scenario, particularly those aspects related to the industrial microbiomes of anaerobic digesters, including microbiome manipulation, big-data and bioaugmentation. The EBA (European Biogas Association), one of the most important bodies linked to the European Biogas Network, is supporting MICRO4BIOGAS in this task.

T1.5: e-Book: This task will see the compilation of an e-Book showing the roadmap of the project through the integration of data collected from all relevant contributions from partners. Summarized reports of each task will be aggregated and complemented with graphical abstracts towards turning the useful information into a user-friendly e-Book that will be publicly available through the project website. Furthermore, this e-Book with the project roadmap will be used for widespread dissemination among potential end-users, policy makers, and biogas plant operators, among others.

Involved partners

The MICRO4BIOGas project is coordinated by Universitat de València (Spain) and comprises the following partners: Gasterra BV (Netherlands), ABS International (Belgium), AEV Energy GMBH (Germany), Ayuntamiento de Aras de los Olmos (Spain), Bioenergie Verbund EV (Germany), Technische Universität Dresden (Germany), Draxis Environmental SA (Greece), Bioclear Earth BV (Netherlands), Universitat Politècnica de València (Spain), Universiteit Gent (Belgium), Finrenes OY (Finland), Darwin Bioprospecting Excellence SL (Spain) and Scienseed SL (Spain).

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This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

Table of contents

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

Figures

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

Tables

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1. State of the Art

Author: Benjamin Rocktäschel, Roland Reiter (AEV Energy GmbH)

Abstract

Agriculture: For biogas production, substrates from agriculture such as corn silage, grass silage, whole plant grain maize, and grain meal are preferred (7). According to the state of the art, these can be digested easily and deliver high biogas yields (9). They are also referred to as energy crops. It makes economic sense to produce renewable raw materials on one's own agricultural land. Unlike the production of food, the whole biomass produced can usually be used here. The resulting digestate can be returned to the fields as fertilizer. This creates a closed nutrient cycle. Through subsidies on the sale of electricity and better utilisation of the cultivated biomass, a biogas plant can contribute to the economic viability of an agricultural company.

Livestock: Manure and dung from cattle and pigs is also a popular substrate for biogas production (4). The process stability is even greater with manure and dung from cattle and pigs, compared to the use of plant biomass. The demands on the process technology are lower. Excreta from poultry such as chickens or turkeys contain a lot of nitrogen (10). These can only be fermented with conventional methods in combination with other substrates. All animal excreta have in common that the expected gas production is lower than when using plants such as corn, grain or grass. Often, residual materials such as liquid manure or dung can be acquired at very low-cost if long transport distances are not necessary. It is even cheaper for livestock farms to use their own residues. Thus, the construction of a small biogas plant often makes economic sense even for smaller livestock farms. The direct use of slurry and manure as fertiliser results in considerable methane emissions, especially in the case of cattle slurry. These can be effectively prevented by digestion in a biogas plant.

Waste disposal: The use of organic waste fractions for biogas production is attractive because waste does not usually have to be paid for. Under certain circumstances, it may even be possible to make a profit from the purchase of waste. Whether the entire process is economical depends on the effort that has to be expended to process the available waste and the methane yield that can be expected. In most cases, pre-treatments such as separation by fraction or shredding are necessary (11). There are special types of digesters for the treatment of stackable substrates. Batch systems such as those presented in section 1.3.1 are particularly suitable for waste (18). Using this technology, pre-treatment can be omitted, and the waste can be freed from organic fractions in this way. The potential for producing biogas from waste varies greatly from region to region. Especially in regions where waste recycling is not widespread, there are often large amounts of waste that can be digested. In these regions, the positive ecological effect is also greatest.

Green Waste: Green waste can be interesting as a substrate for biogas plants if the proportion of lignified plant parts is low. Normally, these substrates are used seasonally and together with other substrates such as energy crops. As long as the dry matter content of the

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material is not greater than about 40 %, the technical requirement is similar to the fermentation of energy crops. Whether it makes economic sense to use it depends on whether a sufficiently large quantity of a constant quality is available, as substrates should not be changed too quickly. With a dry matter content of over 40 % and larger proportions of lignified plant parts, fermenters for stackable substrates can also be used (chapter 1.3.1).

New bioproducts: The primary components of biogas (CO_2 and CH_4) can in principle be separated and further utilised. In the case of methane, this is done by biogas upgrading. The biomethane produced can be fed into the natural gas grid and is mostly used to produce electricity and heat. In the European Union, the production of biomethane has been steadily increasing since 2010. In 2020, 32 TWh of energy was produced in this way (31, 24). CO_2 is also a potential feedstock for syntheses of carbon-containing compounds. However, the cost of carbon dioxide production is too high compared to other chemical processes to make it economically viable. There is also research into the production of other chemical substances during fermentation. However, these are not part of the state of the art.

Wastewater treatment: Sewage treatment produces sewage sludge, which can be treated anaerobically to produce biogas. However, the technical requirements (tanks, agitators, separators, etc.) for this process are fundamentally different from the biogas production described below in the context of agricultural plants. The legal framework conditions are also completely different in most cases. In the case of anaerobic wastewater treatment, the focus is more on the decomposition of organic substances and economic advantages when combined with aerobic processes. Biogas production from wastewater is therefore not considered in the following.

1.1 Introduction

1.1.1 What is biogas

Biogas is a combustible gas mixture and consists mainly of methane and carbon dioxide. Biological processes form the basis for the production of biogas. A combustible gas mixture is produced from organic biomass under anaerobic conditions. The formation of biogas can be observed in natural processes. These take place in bogs, in sediments at the bottom of water bodies and in the faeces and stomachs of cattle (Fachagentur Nachwachsende Rohstoffe, 2013). Biomass is decomposed by various microorganisms in several steps, mainly to carbon dioxide, methane and water. The gaseous components form the so-called biogas. This gas mixture consists of a methane content of 50 to 75 % by volume and a carbon dioxide content of 25 to 50 % by volume (Fachagentur Nachwachsende Rohstoffe, 2013). In addition, it contains other components such as nitrogen, water (as water vapour), ammonia, hydrogen sulphide and hydrogen in small quantities. In the following, only the technical production of biogas is considered. The raw materials of the biomass are referred to as feedstocks. Plants for the targeted and priority production of biogas are called biogas plants. Here, too, biomass is used as the starting material for microbial degradation; the various biomasses are called feedstock. For example, excrement from farm animals, biowaste, residues from food production or energy crops are used (Streicher et al., 2016). The resulting

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biogas can be used in combined heat and power plants to produce electricity and heat, or it can be purified and fed into the natural gas grid. Biogas plants make it possible, especially for companies that have unused biomass available, to save costs for disposal, prevent emissions and generate income through the sale of electricity and heat or gas.

Composition of biogas: The composition of biogas is determined by various influencing factors, which also influence each other. The most important are: the microorganisms involved, the process conditions and the chemical composition of the feedstocks used. The following table (**Table 1**) gives an overview of common gas compositions.

Table 1: Composition of biogas (Fachagentur Nachwachsende Rohstoffe, 2013).

Gas	Concentration
Methane (CH_4)	50–75 Vol.-%
Carbon dioxide (CO_2)	25–45 Vol.-%
Water (H_2O)	2–7 Vol.-% (20–40 °C)
Hydrogen sulphide (H_2S)	20–20,000 ppm
Nitrogen (N_2)	< 2 Vol.-%
Oxygen (O_2)	< 2 Vol.-%
Hydrogen (H_2)	< 1 Vol.-%

Methane – CH_4: Methane is the main combustible component of biogas and therefore the target product in technical production. The higher the methane content in biogas, the higher its calorific value. The more methane is produced, the more electricity and heat can be generated, and thus the more revenue. For utilisation in CHPs, the methane content must normally not be below 40 - 45 %. Under normal process conditions, this methane content is achieved without any problems. Biochemically, methane is formed in various metabolic pathways; it serves as an electron acceptor for the microorganisms involved under anaerobic conditions.

Carbon dioxide – CO_2: Carbon dioxide is the second main component of biogas after methane. Since it is not combustible, it is a by-product from a technical point of view. Carbon dioxide behaves like an inert gas during combustion in the CHP unit and does not contribute anything to the calorific value. If the biogas is to be processed and fed into the natural gas grid, the CO_2 must be separated. Carbon dioxide is formed at many points during microbial degradation. It is formed during hydrolysis/acidification and during methane formation. It is the end point of the oxidative degradation of carbon compounds.

Water – H_2O: Water is found in biogas, mainly as water vapour. The biogas usually has a relative humidity of almost 100 % after formation. In (inactive) combustion engines, this

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moisture can condense and, in combination with hydrogen sulphide, lead to corrosion. Biogas must therefore often be dried.

Nitrogen - N_2: Nitrogen is not formed during the decomposition of biomass under anaerobic conditions, but it reaches the biogas plants in small quantities when air is added. Biochemically, nitrogen behaves inertly and is therefore negligible for the technical process.

Oxygen – O_2: In the presence of oxygen, the energy-rich hydrocarbons in the biomass are completely degraded to carbon dioxide and water without methane being formed. Biogas production therefore takes place in the absence of air. The addition of very small amounts of oxygen with the substrates can hardly be prevented technically, but oxygen is often added to the biogas in a controlled manner to remove gaseous sulphur compounds. Under anaerobic conditions, small amounts of oxygen are consumed very quickly, which is why it is usually only found in very small amounts in biogas. It also generally has an inhibitory effect on methane-forming microorganisms. In larger concentrations, oxygen forms an explosive mixture with methane, which is why the oxygen content is kept as low as possible in technical biogas production.

Hydrogen sulphide – H_2S: H_2S is formed as a gaseous end product of the decomposition of sulphur-containing compounds of biomasses. It is technically undesirable due to its corrosive properties. The content of H_2S in biogas depends strongly on the sulphur content of the outgassing products. Sulphur compounds can have an inhibitory effect on microorganisms. However, such concentrations are not usually reached in agricultural biogas plants. Hydrogen sulphide is toxic to humans, and thus provides an increased hazard potential when coming into contact with biogas.

1.1.2 Biochemical conversion to biogas

In the following, the biochemical basics of biogas formation will be briefly presented. Further information and an introduction to the microorganisms involved can be found in chapter 4. Biogas formation is also known as anaerobic digestion (AD). The anaerobic digestion can be divided mentally into four sub-steps. The process is shown schematically in **Figure 1**. In technical language, all sub-steps are usually summarised as methane formation, since all biochemical processes often take place together in one container. If hydrolysis is separated from the other sub-steps in terms of process technology, acidogenesis, acetogenesis and methanogenesis are summarised as methane formation.

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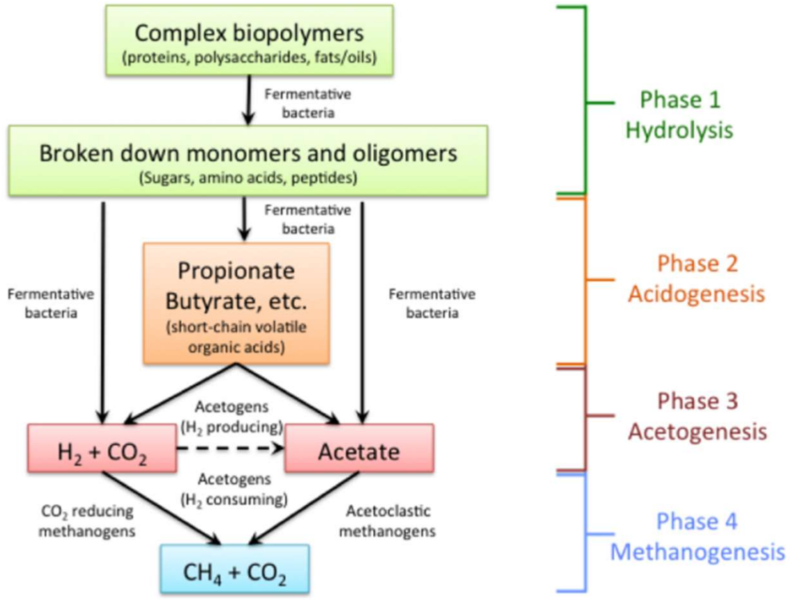

Figure 1: Schematic representation of anaerobic fermentation (*Dutton, online accessed 2021*).

Hydrolysis: The first phase of biochemical conversion to biogas is hydrolysis. In the chemically broader sense, hydrolysis means the splitting of molecules under the reaction with water (Planet Biogas, online accessed 2021). Hydrolysis can take place under anaerobic conditions. Polymers present in the biomass such as cellulose, proteins and fats (complex macromolecules) are split in this phase to oligo-, di- and monomers (shorter split products) (Nwokolo et al., 2020). Bacteria involved in this process release enzymes that biochemically decompose the substrates (Bauer et al., 2009).

Acidogenesis: In the second phase, also called the acidification phase, the hydrolysis products (mainly from the sugars, fats and proteins) are converted into hydrogen, carbon dioxide, alcohols and fatty acids by acid-forming bacteria. The fatty acids are short organic fatty acids such as acetic, propionic and butyric acids (Schnürer, 2016). For the process, it should be noted that ammonia is formed from nitrogen compounds. In excessive quantities, this is toxic for the microorganisms and thus has a process-inhibiting effect (Aberle, 2016).

Acetogenesis: The third phase of anaerobic digestion is acetogenesis (acetic acid formation). The initial products of acidogenesis are converted into even smaller molecules by acetogenic bacteria. Acetic acid, hydrogen and carbon dioxide are formed. A too high hydrogen content inhibits the conversion of the intermediate products of acidogenesis.

Methanogenesis: In the final phase of biogas formation, the acetic acid, hydrogen and carbon dioxide are converted into methane by means of strictly anaerobic methanogenic archaea. The starting materials of methanogenesis are methane, carbon dioxide and water. The production of methane can be divided into two groups. Hydrogenotrophic and acetoclastic methanogenesis. In hydrogenotrophic methanogenesis, methane is produced from hydrogen and carbon dioxide; in acetoclastic methanogenesis, methane is produced by acetic acid cleavage (Schnürer, 2016). The microorganisms involved in methanogenesis are called methanogens. These are light and temperature sensitive.

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- Acetic acid-splitting (acetoclastic methanogenesis): $CH_3COOH \longrightarrow CO_2 + CH_4$
- Hydrogen-utilising (hydrogenotrophic methanogenesis): $CO_2 + 4H_2 \longrightarrow CH_4 + 2\ H_2O$

Different microorganisms are involved in the individual degradation stages, and these make different demands (e.g. temperature, pH value) on the environment. From a process-technical point of view, a compromise must therefore be found which takes into account above all the methanogenic microorganisms. These have a low growth rate and react fragilely to disturbances, so the conditions of the biocenosis must be adapted to their requirements. The biocenosis must therefore allow fermenting bacteria and methanogenic archaea to co-exist. The milieu conditions and operating parameters required for this are discussed below. Depending on the type of feedstock and the design of the biogas plant, different environmental conditions can arise in the individual fermenter stages. The environmental conditions have an impact on the microbial biocenosis and thus influence the metabolic products (Boe et al., 2010).

1.1.3 Milieu conditions

Oxygen: Methanogenic archaea formed about three to four billion years ago. This makes them among the oldest living organisms on our planet. Since the atmosphere had a completely different composition at that time, archaea depend on an oxygen-free environment. Even the smallest amounts of oxygen can have a toxic effect on archaea. An input of oxygen into the fermenters cannot be completely avoided, but this does not lead to the death of the archaea. The archaea live in symbiosis with oxygen-consuming bacteria from the previous degradation steps. These consume the oxygen before it has a toxic effect on the archaea.

Temperature: Although the reaction rate of chemical reactions increases with temperature, the temperature optimum must not be exceeded. There are different temperature optima for the metabolic products of microorganisms (**Table 2**). If these are exceeded or not reached, the processes are inhibited, and the microorganisms can be irreversibly damaged. The temperature optima for biogas plant can be divided into three groups (Dobre et al., 2014).

Table 2: Temperature optima of participating microorganisms.

Thermal stage	Temperature range
psychrophilic	< 25 °C
mesophilic	37 – 42°C
thermophilic	50 – 60°C

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Due to the low temperatures, it is not necessary in the case of psychrophilic microorganisms to heat up the fermenter, but the gas yield per unit of time is significantly lower due to the lower reaction rates. The mesophilic and thermophilic microorganisms have a significantly faster gas production.

The thermophilic temperature range allows a faster gas yield than the other two groups, but it has a lower process stability. Under thermophilic conditions, cultures exist in a lower species range than in the mesophilic group. This means that the fermentation process is more sensitive to the introduction of substrate, the operation of the fermenter and disturbances. The fermenter must also be brought to a high temperature level, which leads to an increase in costs. The most widely used biogas plants are those that operate in the mesophilic temperature range. They offer a good compromise between fast gas production and good process stability.

For the cultures involved, the temperature should be kept as constant as possible. The microorganisms can react and adapt to slow temperature changes, but this is not possible with too high fluctuations in short intervals (Chae et al., 2008). When microorganisms break down carbohydrates, self-heating occurs, which influences the temperature in the fermenter. The temperature can rise to a level of 43 - 48 °C if the fermenter is operated in a mesophilic mode.

pH value: Characteristic for the biodegradation process are the decrease of dry matter and an increase of the pH-value. In principle, the same applies to the pH value as to the temperatures. Different microorganisms require different pH values for optimal growth. The pH optimum of the individual bacteria and archaea is listed in **Table 3**.

Table 3: pH-values Optima of microorganisms involved.

Microorganisms	pH-value
hydrolysing and acidifying bacteria	5,2-6,3
Acetic acid-forming bacteria and methanogenic archaea.	6,5-8

The pH value within a fermenter is automatically adjusted by the biochemical processes. The alkaline and acidic metabolic products formed during anaerobic degradation are decisive for this. The pH value can drop if the acidic metabolic products of acidogenesis accumulate. This is possible, for example, if the substrate supply is too high or if methane formation is inhibited. If the pH value drops, the inhibition effect of hydrogen sulphide and propionic acid may increase, and the fermenter may tip over.

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1.1.4 Inhibitors

Inhibitors can interfere with or delay the processes of the individual degradation stages. In the case of a toxic concentration of inhibitors, these can completely stop the degradation process. Inhibitors can either enter the fermenter via the substrate or they arise as intermediate products of the individual degradation stages. Excessive addition of substrates can also be classified as inhibitory. Microorganisms can adapt to inhibitors, but their adaptability is limited. Examples of inhibitors that are introduced via the substrate include antibiotics, disinfectants or solvents, herbicides, salts or heavy metals, which inhibit the process even at low concentrations (Theuerl et al., 2019). Some of the inhibitors are described below as examples.

Nitrogen / Ammonia is a prerequisite for the growth and activity of microorganisms. If the added substrate is rich in nitrogen compounds (especially proteins), this leads to an increase in the ammonia concentration in the fermenter. Ammonium (NH_4^+) and ammonia (NH_3) are produced during hydrolysis and are in equilibrium with each other.

- $NH_4^+ + OH^- \rightleftarrows NH_3 + H_2O$

An increase in pH increases the NH_3 content. Temperature increases also shift the balance towards the inhibiting ammonia (Fuchs et al., 2018).

Sulphur / Hydrogen sulphide is an essential building block for the microorganisms involved and thus essential for the production of biogas. Dissolved hydrogen sulphide (H_2S) can be toxic and have an inhibitory effect at high concentrations (Aberle, 2016). Elevated H_2S concentrations cause corrosion and can lead to damage in the CHP. H_2S can be converted with the help of sulphur bacteria, which biologically convert the H_2S into harmless sulphur (Aberle, 2016).

- Sulphur hydrogen oxidation: $H_2S + O_2 \longrightarrow S_2 + 2\ H_2O$

1.1.5 The technical production of biogas

An agricultural biogas plant can basically be divided into four process steps (Weiland, 2010):

⇒ Feedstock management (delivery, storage, processing)
⇒ Biogas production
⇒ Digestate management
⇒ Biogas utilization (incl. storage and treatment).

The individual process steps are shown in detail in **Figure 2**. The individual process steps are not decoupled from each other, but are closely connected. For example, process step four provides the required process heat for the second step (Fachagentur Nachwachsende Rohstoffe, 2013).

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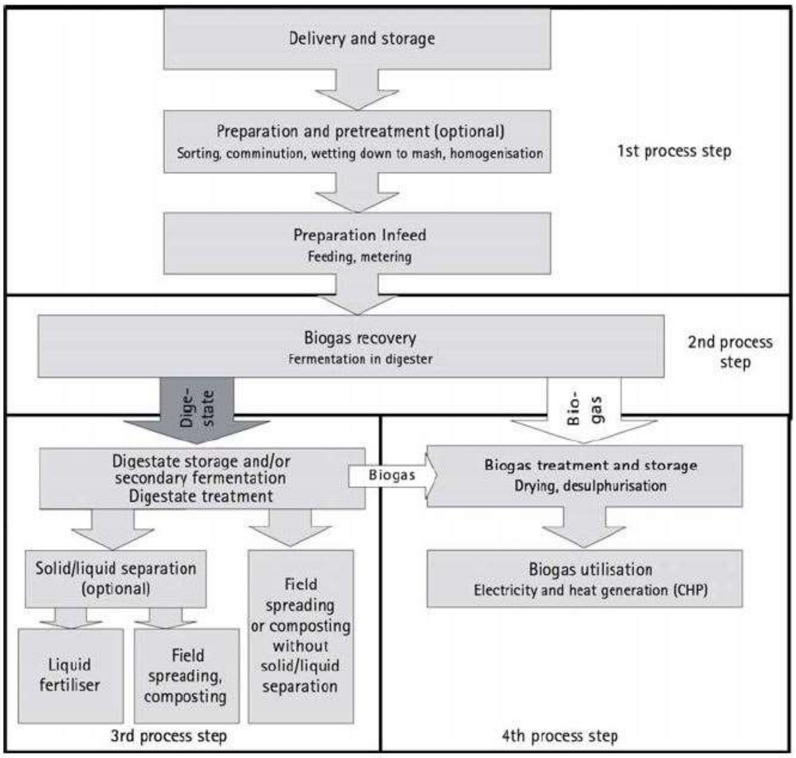

Figure 2: General process of biogas recovery (Fachagentur Nachwachsender Rohstoffe, 2013).

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Components of a biogas plant: The components used in a biogas plant vary depending on the design of the plant. In the following, only the core components of a biogas plant will be discussed. Further information on the individual plant components can be found in chapters 1.2 - 1.5. **Figure 3** shows the schematic structure of a biogas plant.

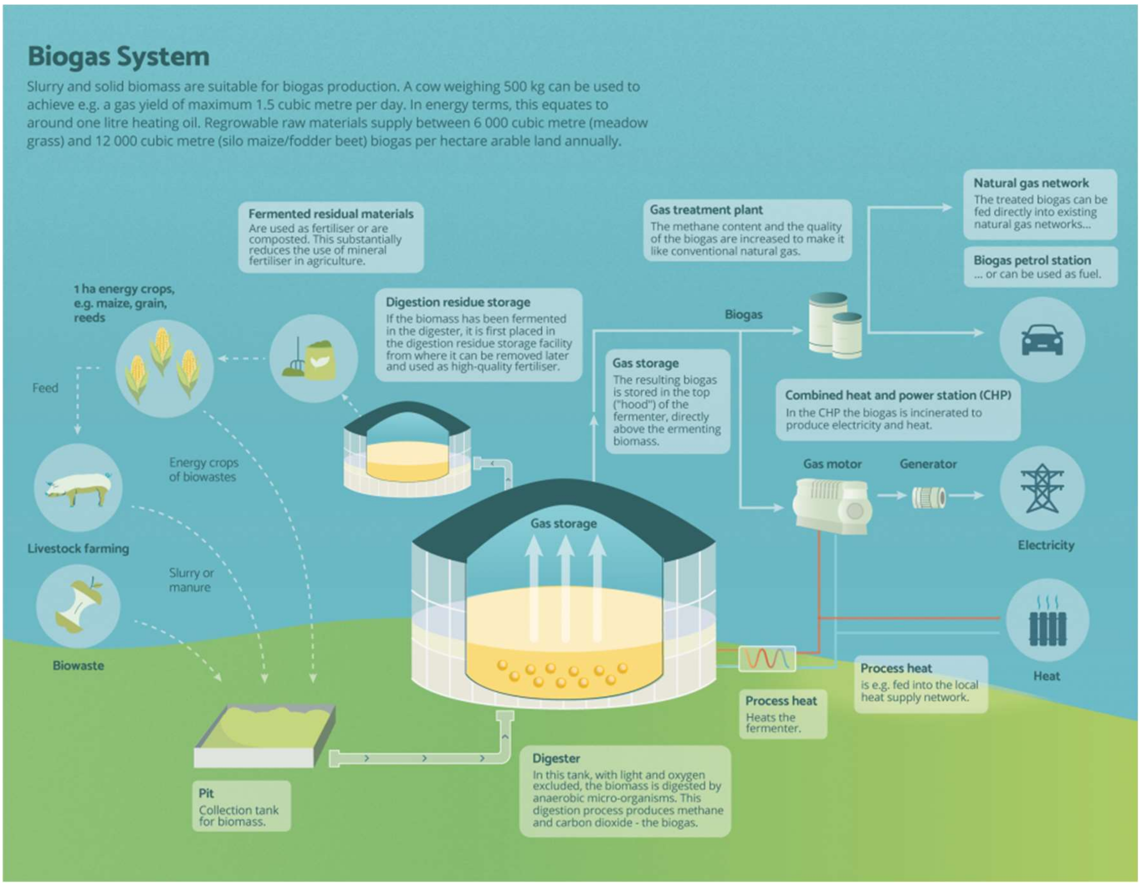

Figure 3: Components of a biogas plant (Planet Biogas, accessed 07.10.2021).

Feedstocks/Substrates: Feedstock are plant and animal substances that can be fermented in biogas plants (Person et al., 2019). They originate from agriculture as well as industry, commerce and municipalities. Feedstocks differ in their degradability and gas yield due to different material properties. In addition to residual materials, renewable raw materials are also used, which are produced specifically for use in biogas plants (Person et al., 2019). The substrates are discussed in more detail in chapter 1.2.

Preliminary pit, silo and receiving feeder: The liquid substrates are temporarily stored in the preliminary pit. These are usually round containers or tanks that are embedded in the ground (Person et al., 2019). The building material is usually ferro concrete, which is often used in the form of prefabricated parts or as cast-in-place concrete. The preliminary pit is located previous to the digester and has the task of storing several days' rations of liquid substrate. The tanks can be open or closed. In addition to liquid, pumpable and agitable substrates, it is also possible to introduce solid substrates to a limited extent. Solid sub-strates are stored in silos. Silos are usually piles of solid substrates that are covered with a

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membrane to protect them from rain. Energy crops (or parts thereof) are stored in silos after harvesting and then continuously removed over the

course of the year. During storage in the silo, lactic acid fermentation occurs. Siloed substrate thus becomes durable and more usable for anaerobic digestion. Solid substrates are conveyed from the silo to the receiving dosing unit using a wheel loader or similar machine. This has the task of conveying the substrates into the plant in the correct quantity and in a suitable form. A receiving feeder consists of a receiving container, a dosing device and often also crushing and weighing technology. The substrates are received either via a chute or a solid's feeder such as a screw conveyor (Person et al., 2019). The comminution of the substrates serves to protect the system from blockages and also significantly increases the usable surface area for the microorganisms.

Fermentation tank/digester: The biochemical degradation process takes place in the fermentation tank. Different microorganisms gradually decompose the introduced substrates under exclusion of oxygen. In the process, biogas is produced in the fermentation tanks. The decomposition takes place under exclusion of air and light. Fermentation tanks are often made of ferro concrete, but constructions made of steel enamel or stainless steel are also common. The airtight cover of the tanks is provided by a flat solid or foil roof with integrated gas storage. To facilitate the heating of fermentation tanks, they are usually insulated. Heating is usually provided by the waste heat from a CHP unit. Systems for mixing, usually agitators, are installed in the tanks; these prevent differences in concentration and temperature within the fermentation substrate. In doing so, they mix the bacteria and archaea in the tank with the fresh biomass. Agitators also prevent sedimentation and flotation of the fermentation substrate and, depending on the viscosity, are also important for gas discharge.

Secondary digesters: In two-stage processes, a secondary fermenter is connected downstream of the fermentation tank/digester. This is where substrates that are more difficult to degrade are converted. A secondary fermenter is often necessary to meet the requirements for a minimum retention time in the gas-tight system and thus avoid emissions.

Separator: The digestate from the fermenter is separated into a solid and a liquid phase in the separator. The liquid phase is collected and discharged as liquid fertiliser after intermediate storage. If required, it can also be used to mash the solid substrates in the fermenter. The solid phase has a reduced volume and can be used as a highly concentrated fertiliser.

Digestate storage tank: The digested fermentation residues are collected in the digestate store. These are then used in agriculture. Digestate tanks are located downstream of the fermenter and the separator. Depending on the intended use, the digestates are stored in different ways (Effenberger et al., 2008). The digestate storage must be large enough to store the digestate for the next thinning in spring or autumn. The liquid phase of the digestate is stored in round basins or lagoons, the solid phase on paved areas provided for this purpose.

Gas storage: Gas storage tanks are used to buffer fluctuations in gas production and to store biogas for flexible operation. This enables flexible operation of the CHP units, which then do not have to be operated permanently, but can also be shut down for several hours. The plant can thus be operated according to demand and feed in electricity when it is needed and best remunerated. In the event of technical faults at the CHP unit or during maintenance work,

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gas storage facilities prevent the loss of biogas for a limited time. Gas storage tanks, as well as all gas-tight covered fermentation tanks, are equipped with an over-vacuum safety device. In the event of overpressure, they release biogas into the atmosphere and in the event of under pressure they let in air. This is to prevent damage. Safety shutdowns, operating methods and the gas flare must ensure that a release of biogas through the overpressure safety devices occurs as rarely as possible.

Heat reservoir: Heat reservoirs are water tanks with an insulating layer. They store generated heat even during times when no CHP is in operation. The stored heat can be used, for example, to heat the fermenter or to dry substrates. Heat reservoirs are necessary if the CHP units are not to be run for a longer period of time, i.e. if they are to be operated flexibly.

Gas flare: A gas flare is almost always connected to the gas pipe system of a biogas plant. This burns biogas in an emergency to prevent uncontrolled release in the event of overpressure. This is to prevent emissions and explosive atmospheres on the plant.

Combined heat and power plant (CHP)/ gas utilisation: The utilisation of biogas usually takes place in so-called combined heat and power plants. There, electricity and heat are generated from the biogas. Gas utilisation is mostly carried out by means of gas Otto engines with flange-mounted generators. Some of the thermal and electrical energy produced is returned to the biogas plant or fed into the public grid. The thermal energy produced is used in the fermenter, temporarily stored in buffer tanks, fed into a district heating network or used for drying plants. If heat utilisation is not possible (e.g. in summer when the heat demand is low), the CHP units have emergency coolers that dissipate the excess heat to the ambient air. The electrical energy is fed into the public grid via a transformer station.

1.2. Substrates

1.2.1 Feedstocks for biogas production

The composition of the substrate mix and the nature of the individual substrates (dry matter content, structure, origin, etc.) determine the design of the process technology (DBFZ Deutsches Biomasseforschungszentrum, 2021). The biogas yield is not only substrate-specific, as one could assume based on **Figure 4** shows, but it also depends on the boundary conditions such as HRT, temperature and the plant operation. Therefore, different gas yields can occur with the same substrate input.

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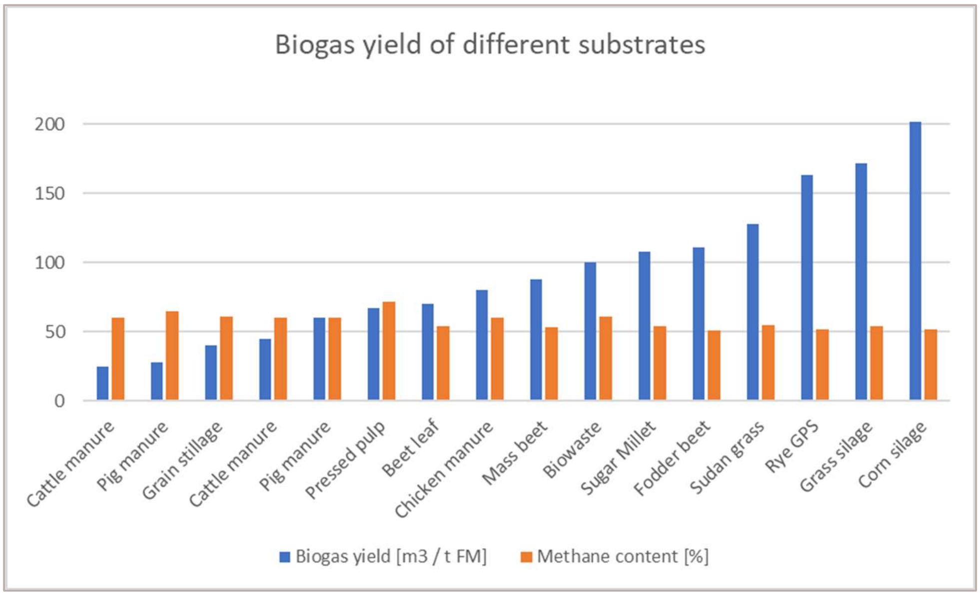

***Figure 4**: Biogas yield of different substrates (Bekon GmbH, accessed 18.10.2021).*

When using renewable raw materials, the operator should make use of different feedstocks to be able to react to crop failures and price fluctuations. In Germany, biogas plants are often operated primarily based on animal by-products such as liquid or solid manure and renewable raw materials (Nwokolo et al., 2020). Substrates can be differentiated according to their material properties, degradability, and gas yield. The methane yield of the substrate depends on its composition and the content of protein, fat, and carbohydrates (see **Table 4**).

***Table 4**: Estimation of the maximum theoretical methane yield and biogas percentage composition (Nwokolo et al., 2020).*

Nutrient	**Methane Yield (m^3/kg VS)**	**CH_4 [%]**	**CO_2 [%]**
Carbohydrate	0.42	50	50
Protein	0.50	70	30
Lipid	1.01	70	30

The lifetime of a biogas plant also depends on the substrate used. Material with a high protein content increases the concentration of H_2S, which is harmful to humans and machines as well as (above a certain concentration) to the biogas-producing microorganisms. Monitoring the gas composition with different substrates is therefore necessary (Effenberger et al., 2008).

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Species and origins of different substrates: The origin of substrates according to (Persson et al., 2019):

From agriculture:

- Animal farming (farm manure/animal excrement)
- Crop production - cultivation (renewable raw materials such as corn, cereals, suda grass, sweet sorghum)
- Crop production – residues (grain plaster, straw)

From industry, commerce, municipality

- Food processing industry (production waste, incorrect batches, returns, wastewater)
- Food trade (market waste, superimposed food)
- Food processing industry (gastronomy, catering)
- Municipal waste management (bio bin, green waste, agricultural care, residual waste)
- Special industrial sectors (pharmaceutical industry, food supplements)

Animal by-products are divided into three categories. Category one material may not be used in biogas plants. This includes animals suffering from mammalian diseases, pets, zoo and circus animals, kitchen and food waste from airports. Category two and three materials may be introduced into biogas plant. Category two includes manure, dung, stomach and intestinal contents, colostrum and inhibitor milk. Manure fermentation makes a major contribution to reducing greenhouse gas emissions, not only due to the production of biogas, but especially due to the reduction of CH_4 emissions from open storage (Weinrich et al., 2020). The use of plant by-products such as straw and crop residues in biogas plant can be economical and leads to process stability when used together with ABP. This reduces the ammonia content and ammonia inhibition (Fuchs et al., 2018; Verein Deutscher Ingenieure, 2006). Plant by-products include brewer's grains, waste bread, starch, molasses, peels, fruit and vegetables and spoiled fodder silage.

For waste, water content and degradability are important factors in the selection of suitable substrates. Fermentable materials that are too moist for composting can be used for digestion. Residual waste comes from private households or public places. The organic fraction in residual waste can be separated using sieves, air sifters and metal separators and transported to the Biogas plant for further processing (Nwokolo et al., 2020). Organic bins are taken to the Biogas plant as separated organic material. Garden and park waste is collected in green waste containers at decentralised collection points and transported to the Biogas plant. Municipal sewage sludge is obtained from the various purification steps of wastewater treatment; this can be recycled in Biogas plant. It should be noted that sewage sludge may contain antibiotics, which have an inhibitory effect on methanogenesis. Restaurants, kitchens and canteens collect food waste in bio bins or containers, liquid waste from beverage

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production and sludge from industrial processes is collected in tanks. This type of waste requires a high level of processing, for example removal of packaging, but provides a very high biogas yield.

1.2.2 Delivery and storage of substrates

Delivery: Substrate preparation is the first process step in biogas production. In Biogas plants that are integrated into agricultural or food-producing operations for the utilisation of residues, the transport is carried out internally. If substrates are produced off-site, they are delivered in substrate-specific containers. The quantity and quality of the substrates are recorded in the delivery area (Persson et al., 2019).

Non-farm liquid substrates are delivered in tankers, liquid manure drums and silo vehicles. Weighing is one way of recording quantities; alternatively, the incoming material flow can be measured by means of a volumetric flow meter. The closed construction of the transport vehicles reduces odour emissions. Filling the substrate also produces few odour emissions, as it is pumped into closed receiving containers via a hose connection. In the case of open unloading processes, e.g. intake via an open bottom flap, the closed system (transport and storage) is interrupted and odours are formed (Nwokolo et al., 2020).

The delivery of solid substrates depends on the type of substrates. Bulk agricultural substrates are transported using standard agricultural transport technology. Other bulk substrates, e.g. biowaste, are transported in container vehicles, foodstuffs can be delivered on pallets or in cartons. The simplest way to record incoming substrates from outside the farm is to count the delivery vehicles at defined transport volumes, which allows a rough estimate of the amount of material. Precise measurement of incoming substrates is ensured by weighing equipment. These can be external or installed directly in the biogas plant. Silage can be recorded after storage by measuring the silo body. Agricultural substrates are mostly transported in mobile silos; the resulting odour emissions and dust developments can be considered marginal. Agricultural substrates that have to be stored dry are stored in high silos (Koch et al., 2017).

Storage: The storage of the substrate serves to keep the necessary quantities for feeding. The substrates should be stored with the lowest possible energy losses and without any harmful impact on the environment. There are great differences in local rules and regulations, especially with regard to proper storage in terms of emissions, which must be taken into account during planning.

The stored biomass contains large amounts of chemical energy. For the full utilisation of a plant with a rated electrical output of 500 kW, about 9,500 tonnes of corn silage must be stored (Amon et al. 2015). For substrate storage in biogas plants, there are solid manure storage facilities, silage camps or flat bunkers, tower silos (dry bulk silos), tower silos for moist preservation, foil tube silos, bale silos, discharge bunkers and reception halls (Persson et al., 2019). In the following, the receiving halls, the silage camps and the tower silos will be dealt with in more detail in this paper. As an alternative to storage on the farm premises,

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temporary silo facilities can also be erected directly on the field. However, these field silos are not permissible in many areas, as degradation products are released unhindered into the environment.

Receiving halls: Halls for receiving substrates are technically complex and cost-intensive; they are only used in very special cases, such as when substrates release special emissions or may not be stored openly. This can be the case with waste, for example. The storage area is determined by the expected substance quantities and storage periods. If problematic substances are delivered as cosubstrates from industrial origin, they must be stored in a receiving station separate from the agricultural operation. Before hygienization of the corresponding substrate, it must not be mixed with harmless substrate. To minimise emissions, the storage should be closed. This can be done, for example, in halls that contain the reception and preparation of the substrate. The exhaust air should be able to be discharged in a targeted manner and, if necessary, fed into an exhaust air purification system. To prevent odours from escaping, such halls are equipped with a negative pressure system which, in addition to extraction, prevents odours from escaping. Halls offer the advantage that the technology used is protected from the weather, and the same applies to the performance of maintenance work. The enclosure in halls makes it possible to comply with noise protection legal requirements and regulations. The employees in the halls must be protected from hazardous gases, this applies in particular to hydrogen sulphide (H_2S). Protection can be achieved through the targeted discharge of gases from storage containers before staff access and through technical equipment such as warning alarms (Fachagentur Nachwachsende Rohstoffe, 2013).

Silage camps: Silage camps are surface structures that are open to the atmosphere and used in biogas plants. Silage camps are used as storage for stackable biomass and bulk material. With regard to the planning requirements, the quality assurance of the silage must be ensured, and the negative environmental influences must also be minimised as far as possible. The Silage camps system should be designed in such a way that the stored biomass can be sufficiently compacted. It must be possible to seal the biomass airtight by means of a cover. Fermentation juice/leachate (produced by hydrolysis and lactic acid fermentation) and rainwater must be able to be discharged separately in some cases. Biomass losses due to leachate formation should be minimised, no biomass should be discharged uncontrolled into water bodies. The silo walls cannot be built to any height, as the amount of fermentation juice increases significantly from a stack height of five to six metres. For high silage quality, professional placement of the biomass is indispensable, as is ensuring sufficient compaction and covering the silage with a suitable film. It is recommended to divide the silos into chambers, which facilitates the professional and low-emission management of the silos. To allow good access, it is recommended that the chambers measure at least four times the width of the vehicle in the transverse direction. The gating surfaces should be designed to minimise solar radiation. The silo plate must be liquid-tight and resistant, no surface water from the surroundings may flow into the plate. Fermentation juice, leachate and contaminated rainwater must be collected within the floor slab and must not flow next to it. The walls must be impermeable to liquids. Fermentation juice, leachate and contaminated precipitation water are collected in a container and pumped from it into the fermentation tank or into the digestate storage tank (Postel et al., 2009).

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Figure 5: Silage camps (Alberle, 2016).

Tower silo: In addition to agricultural use, tower silos are also used in the biogas plant sector. They are usually placed outdoors, but smaller containers can be placed in halls or buildings. For biogas plants, they serve as storage for grain, maize grains or corn-cop mix (CCM). They serve as a template for the addition of energy-rich substrate. They are less common than mobile silo systems. A distinction is made between dry silos and high silos for moist preservation. In ventilated dry silos, relatively dry substrates such as cereals and grain maize are stored, while in high silos for moist preservation, substrates such as crushed grains are mixed with water and preserved as a pumpable slurry under exclusion of air. High silos can be considered emission-free. The equipment often consists of cylindrical containers with bolted steel segments with a roof, extraction hopper and the conveying equipment (Persson et al., 2019). Alternatively, the container materials consist of plastic reinforced with glass fibre, fabric or concrete (Persson et al., 2019). For the construction of high silos, it is essential to build a load-bearing foundation. Blowers, screw conveyors, elevators and pumps act as filling and removal equipment. In addition, elevated silos have crushing technology and thus have a high degree of automation with regard to the storage of the substrate. The conveyor technology transports the substrates directly into the fermenter or into a feed tank/mixing tank. High silos can optionally be attached to weighing cells; this is helpful for precise metering and level measurement. The advantage of this plant component is the low space requirement and the highest possible degree of automation, which results in low labour costs when using high silos. The disadvantage of dry silos is that the storage time depends on the moisture content of the grain and the high purchase costs.

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Figure 6: Tower silo

1.2.3 Substrate feeding

Distinction is made between pumpable substrates such as liquid manure and stackable substrates such as corn silage. Substrates with a low dry matter content (DM content) of approx. 4 - 15 % are particularly suitable. At less than 4 %, economic utilisation by means of mono-digestion is difficult, as much non-utilisable water is contained. Above 15 %, the substrate usually has too high a strength, so that the stirring and pumping ability is no longer given. The feed/feeding of a biogas plant is the supplier of fresh substrate for the microorganisms. This can be done continuously, quasi-continuously and discontinuously. Substrate feed, for example, can be carried out via feed systems such as a solid's feeder with elevators, feed screws, feed shafts, preliminary pits and pipelines.

Liquid feedstocks: The following criteria must be observed when dimensioning pumps (Kissel et al., 2014):

- Medium (composition, dry matter content, pH value and temperature).
- Geodetic transport height.
- Pipeline (diameter, length and number of fittings).
- Volume flow.
- Tank (diameter and height)

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The substrate feed is usually carried out with a pump for liquid substances. The following pumps are usually used (**Figure 7**):

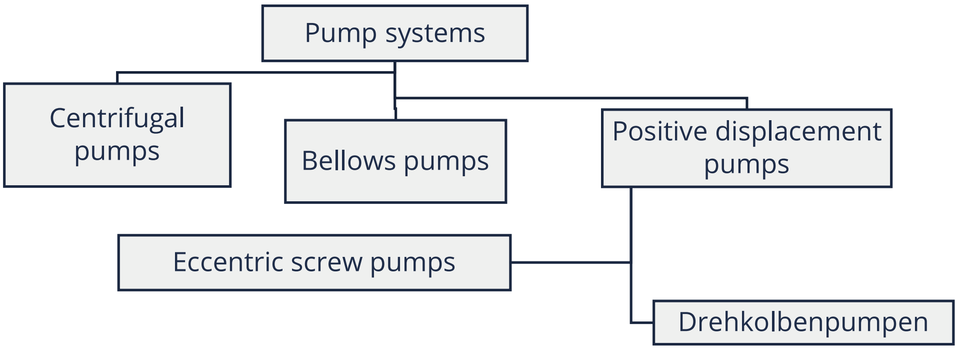

Figure 7: Pumps for liquid feeding

Only small amounts of solids may be conveyed in the process. The substances are added via a preliminary pit. A rod mixer or a submersible motor propeller unit is usually installed in this. Ideally, the digester should be fed continuously; this guarantees a stable fermentation process. In practice, the fermenter is fed quasi-continuously, in short time intervals at regular intervals, with a feed quantity that is as constant as possible. The pumping process is usually fully automated. Slurry contains litter as well as feed residues, so the pumps for substrate delivery must have special characteristics (Biomin, accessed 18.10.2021). In agricultural biogas plants, mainly centrifugal pumps and positive displacement pumps are used to convey the liquid input material. To protect the pumps, they are often preceded by cutting and crushing devices and foreign body separators. The pumps must have a high capacity to avoid clogging. **Table 5** shows the design of the pumps and their characteristics in comparison.

Centrifugal pumps have a relatively simple technology, they are robustly built and are used for substrates with a dry matter content of 0 % - 12 %. The delivery rate of this type of pump depends on the delivery head. The flow rates vary between 2 and 6 m^3/min, with a power consumption between 3 and 30 kW (Effenberger and Lebuhn ,2011).

Positive displacement pumps are used when viscous materials need to be pumped. The quantity pumped can be determined by the number of revolutions. This theoretically increases the process stability and the control of the system. Positive displacement pumps are more susceptible to faults than centrifugal pumps; it is advisable to connect a macerator in front of the pump to protect it by crushing to coarse organic matter (Roskatski, 2019).

Bellows pumps are particularly suitable for viscous pumpable substrates with high levels of impurities (Roskatski, 2019). They are more robust than positive displacement pumps and insensitive to dry running, but only pump very small quantities.

Eccentric screw pumps (see **Figure 8**) can also be used to pump viscous substrates; in contrast to bellows pumps, only a small proportion of impurities may be present. Furthermore, eccentric screw pumps are sensitive to dry running. However, they can self-primate from a

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depth of up to 8.5 m and reach a discharge pressure of 24 bar; the delivery rate is generally lower than that of centrifugal pumps (Kissel et al., 2014).

Rotary lobe pumps can also be used; they are less sensitive to impurities than the eccentric screw pump, have a high delivery rate and are characterised by a simple design (Biomin, accessed 18.10.2021).

Table 5: Pump comparison (BioBG, accessed 25.10.2021).

Type	**Centrifugal pumps**	**Positive displacement pumps**
Construction methods	Submersible centrifugal pump	Eccentric screw pump
	Centrifugal immersion pump	Rotary lobe pump
Properties	high flow rate, low pressure, not basically self-priming	meterable, self-priming, constant pressure, also for viscous materials

Figure 8: Eccentric screw pump of the company Armatec FDM GmbH & Co. KG (accessed 13.10.202).

Solid feedstock: The solid substrates can also be introduced via a feed or conveying screw. In this case, the solid material is pressed below the liquid level in the fermenter by tamping screws. This prevents gases from escaping (**Figure 9)**.

Another possibility is to introduce the solids by means of a piston (**Figure 9)**. A hydraulically operated feed cylinder presses the substrates into a pressure channel that is below the liquid

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level of the fermenter (Hopfner-Sixt, et al., 2007). After the substrates have been introduced, the channel is closed again by a hydraulic slide valve. The introduction of the solids by means of feed pistons enables automatic dosing at any time intervals. With this method, there is a risk that the substrate introduced will form a sink layer and clump together, which means that it is no longer optimally degradable for the microorganisms.

Containers with and without crushing tools are used to feed the feed screws and feed pistons (Kissel et al., 2014). Size reduction increases the substrate surface for microbiological degradation, which has a positive effect on methane production. The higher the comminution, the faster the degradation takes place, but the gas yield does not necessarily increase.

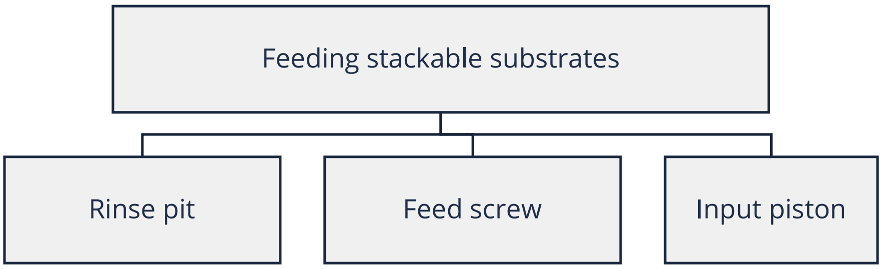

Figure 9: Feeding of stackable substrates

1.3. Anaerobic Digesters

The digester is the main component of a biogas plant. In this reactor, microorganisms convert organic biomass into biogas. The digester must provide optimal growth conditions for the microorganisms to ensure the highest possible biogas production. Essentially, a digester consists of a fermentation tank, which is thermally insulated, a heating system, mixing units and, if necessary, discharge systems for sediments and the fermentation substrate (Fachagentur Nachwachsende Rohstoffe, 2013). Biogas plants can be divided according to the type of fermentation process into continuous wet fermentation, continuous dry fermentation and discontinuous dry fermentation (Effenberger and Lebuhn, M., 2011). In 2016, an operator survey (reference year 2015) was conducted from 310 samples (Nwokolo et al., 2020). The result of the survey can be seen in Figure 8. The most commonly used reactor types were stirred tank fermenters, followed by multistage systems in which the stirred tank fermenter is followed by another reactor type (usually as a secondary fermenter). With a three percent share of the distribution of fermenter systems, the plug flow reactor was named second most frequently. Newer reactor types such as ring-in-ring solutions or those operated according to the double-chamber process (Pfefferkorn principle) were only rarely mentioned. The small number of special processes can be attributed to the low rates of expansion of biogas plants after their market maturity (Fachagentur Nachwachsende Rohstoffe, 2013).

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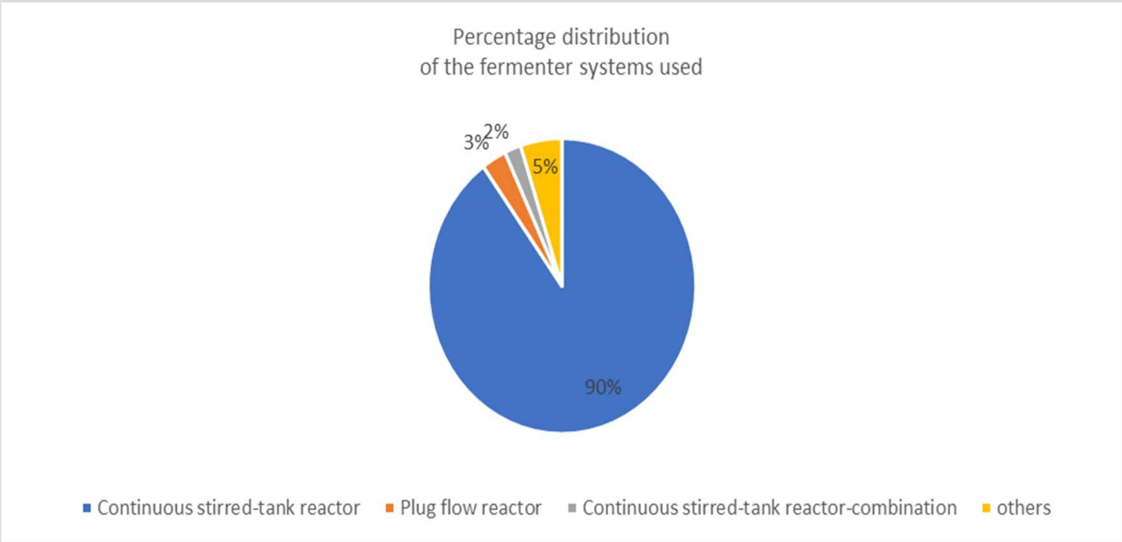

Figure 10: Percentage distribution of the fermenter systems used (operator survey 2016, reference year 2015) (Planet Biogas, accessed 07.10.2021).

1.3.1 Types and designs of containers

General construction types of containers: Fermenter designs can be differentiated according to their geometry (round, angular, horizontal, or vertical), their mixing (stirred tank, plug flow), their process requirements (parallel, serial), their biological arrangement (single-stage, multi-stage) and their constructional design.

In digesters with complete mixing, the substrate fed in is distributed evenly over the entire contents of the digester. When fresh substrate is added, the corresponding amount of fermentation substrate is removed from the reactor.

With plug flow, the substrate is fed to the beginning of the reactor. A defined amount of substrate fed in will theoretically not mix with another defined amount until the end of the reactor. In practice, however, this does not apply due to recirculation flows.

In batch digesters, neither mixing of the biomass nor continuous feeding of further substrate is necessary. **Table 6** shows a comparison of the properties of different fermenter technologies.

Biogas reactor with complete mixing (storage-flow process): As can be seen in **Figure 11**, stirred tank fermenters are the most common type, with a share of 90 percent. They are continuous stirred tanks and are mainly used in the field of agricultural biogas production. They are fully mixed reactors in cylindrical upright design.

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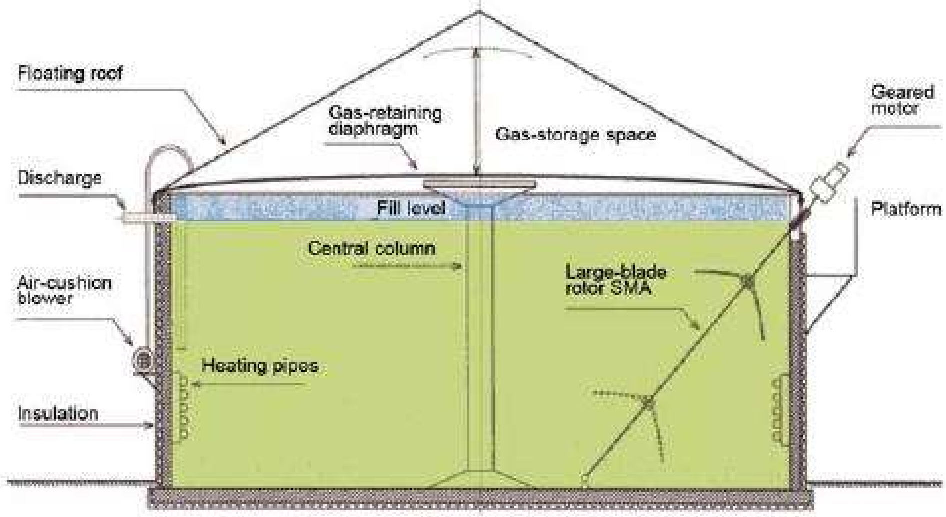

Figure 11: Stirred tank reactor with long shaft agitator (Energypedia, accessed 15.10.2021).

The reactor is characterised by its simple, robust and reliable technology (Weiland, 2010). The tanks are designed to maximise the contact between the biomass and the substrate, which increases the fermentation performance. Feeding is automated to the greatest possible extent. The digester is designed as a tank with a concrete floor and walls made of steel or ferro concrete; it can be fully or partially sunk into the ground or built completely above ground (Mutungwazi et al., 2018). Foil roofs or concrete ceilings are built on top of the tanks for gas-tight covering. Mixing is achieved with one or more agitators located in or on the reactor. The agitators are arranged either centrally or laterally. Hydraulic fermenters without agitators are also possible. The mixing devices must be very efficient. Agitator systems are discussed in more detail in chapter 1.3.3. A size of 6,000 m^3 is possible; as the size increases, mixing and process control become increasingly demanding. In principle, all substrate types can be introduced, ideally pumpable substrates with a low to medium dry matter content. This type of fermenter is basically suitable for continuous, quasi-continuous and discontinuous feeding. Advantages are the low-cost construction, the variable operation as flow-through or according to the flow-through-storage method and the maintenance of technical aggregates can be carried out without emptying the fermenter. Disadvantages are the high costs due to the energy-intensive agitators, short-circuit flows (parts of the fresh substrate can get into the outlet) are possible and floating blankets and sink layers can form (Fachagentur Nachwachsende Rohstoffe, 2013).

Plug flow biogas reactor: Plug flow fermenters are designed in the form of horizontal pipes with a round or rectangular cross-section. They use the displacement effect of the substrate to achieve a plug flow through the fermenter. The substrate is continuously fed into the reactor via the substrate feeder and the feed device, usually a screw. The feed device creates a pressure that guides the substrate through the reactor chamber. The plug flow leads to a separation of the fermentation stages within the tank. The schematic construction is shown in **figure 12**. The agitators are used to mix the substrate. They use special agitators, mostly slow-running paddle shafts, to cause mixing across the direction of flow (direction of movement of the substrate), but not in the longitudinal direction. There are horizontal and vertical

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plug-flow fermenters; horizontal reactors are mostly used in agriculture. Standing reactors are rarely used; they also exist in designs without agitators (Veluchamy et al., 2019). They use gravity and pumps to mix the biomass.

Fermenters of this type are mostly made of steel or stainless steel as well as ferro concrete. The size of horizontal digesters is about 800 m^3 for horizontal digesters and 2500 m^3 for vertical digesters. For economic reasons, horizontal reactors are usually only manufactured up to a volume of 300 m^3; in these size regions, the advantages of the compact and cost-effective design outweigh the disadvantages. The fermenters are built in the factory and transported to their place of use, which limits the size of the tanks (Fachagentur Nachwachsende Rohstoffe, 2013).

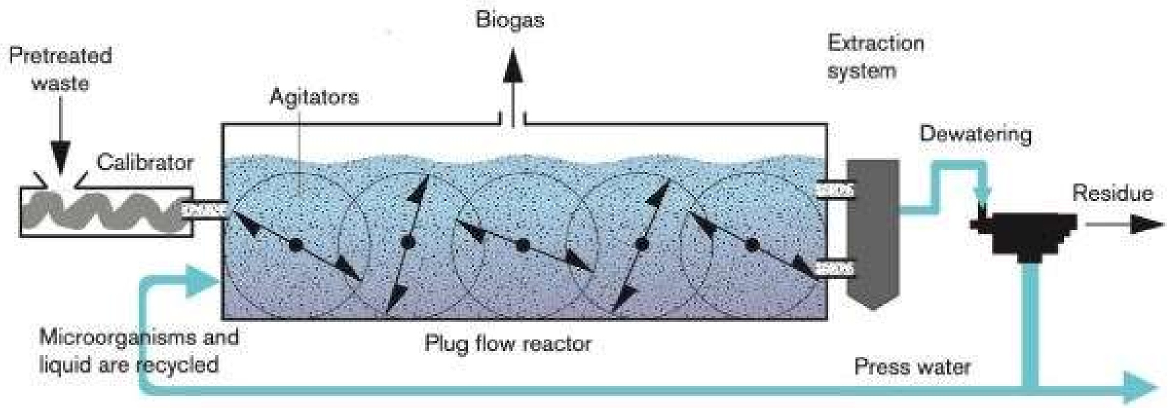

Figure 12: Plug flow reactor (Persson et al., 2019).

The tanks are thermally insulated, often offer several devices for gas extraction, substrate connections as well as sediment discharge, agitator and heating pipes. Heating coils can be integrated into the paddle agitators.

Biogas reactors with plug flow are suitable for wet fermentation, mostly pumpable substrate with high dry matter content is used. Quasi-continuous or continuous feeding of the tanks is envisaged (Veluchamy et al., 2019). The compact design is an advantage, which is why these digesters are preferably used in small plants. Due to the construction, floating ceilings, sinking layers or sedimentation of solids are usually avoided. The retention times are usually adhered to and can be determined relatively accurately, as short-circuit flows cannot occur as with other reactor types. Due to the small size, there is less heat loss, and the reactor can be heated effectively. The small size of the fermenters is a disadvantage, and the fermenter must be completely emptied for maintenance work. The process stability is lower than with stirred tank fermenters, and the operating and construction costs are usually higher.

Biogas reactor according to the batch process: For discontinuous operation, so-called boxes or garages are used. These can usually be opened via a gate and emptied and filled directly. The box fermenters are made of ferro concrete or steel. In practice, several containers are connected in parallel and operated at different times to be able to operate the downstream plant continuously. Two to eight units, usually four units, are used in parallel. This

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enables quasi-continuous gas production. Box digesters are particularly suitable for dry fermentation, often using pourable substrates such as corn and grass silage. This type of reactor is particularly advantageous for substrates that cannot otherwise be handled, such as organic waste with impurities. **Figure 13** shows the process of a box fermenter from BEKON GmbH.

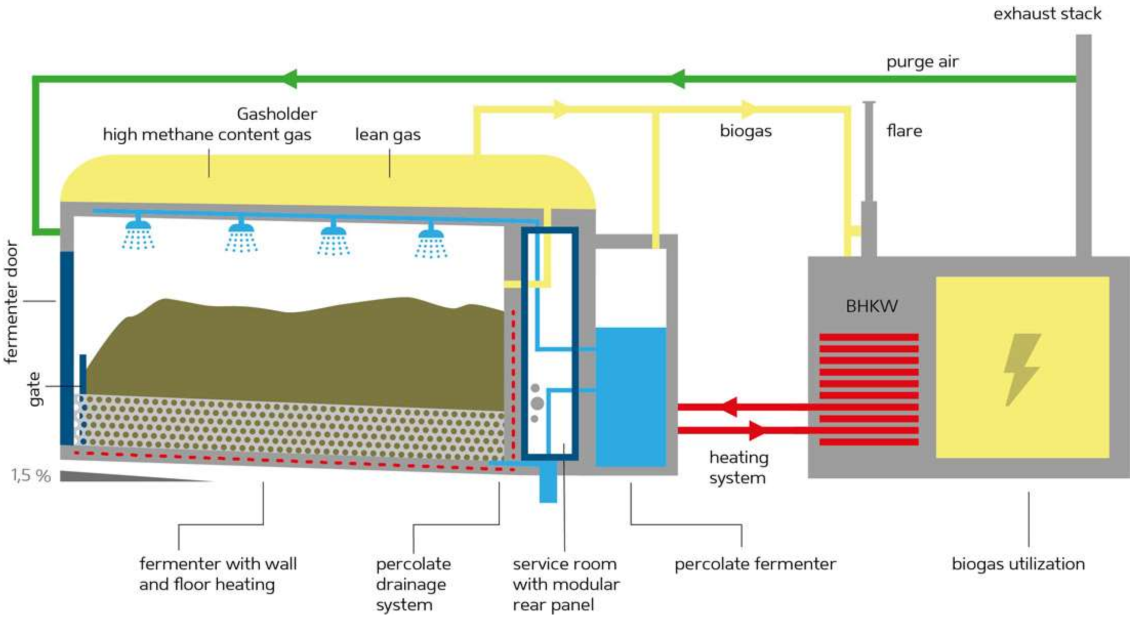

Figure 13: THE BEKON PROCESS, Process of a box fermenter (Bekon gmbH, accessed 18.10.2021).

In the batch process, the fermenters are filled with substrate, sealed airtight and only opened after outgassing. Both the filling process and the emptying are usually carried out by means of a wheel loader (Clarke, 2018). The ratio of fresh material to inoculation material is 40 % to 60 %. This ensures that the substrate is supplied with sufficient anaerobic bacteria. For humidification, the substrate is sprayed with percolate (leachate of the substrate, which is recirculated) with nozzles attached to the ceiling throughout the entire residence time. The percolate migrates through the substrate, is collected and pumped into a storage tank. The temperature of the biomass is controlled by means of attached wall and floor heating. Gas collection pipes are attached to the fermenter, in which the biogas is collected and discharged (Fu et al., 2018; Weiland, 2010).

Box digesters are usually single-stage processes in batch operation, the various degradation reactions (hydrolysis, acid and methane formation) take place in one process step.

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Table 6: Properties of different fermenter technologies (Weiland, 2010).

Reactor	Feeding	Temperature	Stirring	Substrate	Reliability
Stirred tank reactor	continuously	Mesophilic or thermophilic	Flow-through fermenter with agitators, hydraulic fermenter without Agitators	Easy to pump, Use for various substrates	Foreign substances can cause technical problems
Plug flow reactor	continuously	Usually thermophilic, also mesophilic possible	Longitudinal or transverse to the flow, vertical systems without agitators	Pumpable, mainly used for municipal biowaste	High tolerance to Foreign substances
Box fermenter	dis-continuously	Mostly mesophilic, also thermophilic possible	No agitators, Percolation system	Stackable, mainly used for municipal organic waste	Robust fermenter without moving parts

Double chamber process (Pfefferkorn principle): Another approach to wet fermentation is the use of double-chamber processes. A biogas plant based on the Pfefferkorn principle (after the Austrian inventor Herbert Pfefferkorn) is a plant that works without an agitator in the fermenter. The reactor contains a main fermentation tank with an enclosing secondary fermentation tank with hydraulic mixing. This process eliminates the need for agitators, which saves electrical energy. In addition, the maintenance effort is reduced, as no agitator units have to be changed or serviced, which also saves costs. The substrate used consists mainly of liquid manure and small amounts of corn silage and grain meal. The construction effort is much higher than with stirred tank fermenters. The fermenter volume is between 400 and 6,000 m^3 (Fachagentur Nachwachsende Rohstoffe, 2013). Mixing takes place through pressure surges, which lead to flows. The pressure surges are generated when the gas at the end is not extracted but displaces the liquid phase and thus leads to different filling heights of the two tanks. Due to a sudden pressure equalisation, the filling levels then balance out abruptly. A major disadvantage of the system, apart from the high construction costs, is the enormous technical effort required to deal with any blockages that may occur.

1.3.2 Construction types of the tank heaters

To ensure a stable fermentation process, there should be no temperature fluctuations in the fermenter. Temperature fluctuations must therefore be kept low, both in terms of temporal

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temperature fluctuations and the temperature gradient within the fermenter. Excessive fluctuations as well as exceeding or falling below temperature limits can inhibit the process or even cause it to come to a standstill. According to the German agency for renewable resources (Fachagentur Nachwachsende Rohstoffe, 2013), temperature fluctuations can lead to:

- The supply of fresh (cold) feedstock
- Temperature stratification or temperature zone formation due to insufficient thermal insulation, ineffective or incorrectly dimensioned heating, insufficient mixing
- Location of the heaters
- Outside temperatures
- Failures

External or internal heat exchangers or heaters provide the required process temperature. They compensate for heat losses and heat the substrate. Accordingly, heating technologies can be divided into system-integrated and external systems.

Integrated heating: For the integrated heating solutions, a distinction can be made between wall heating, floor heating and heated stirring unit. Wall heating is carried out by installing heating pipes inside the tank in the wall as well as on the outer wall (Persson et al., 2019). Stainless steel pipes, PVC or PE are used as material for the pipes. Stainless steel pipes have a better heat transfer than plastic pipes, turns can be saved, but they are also more expensive. In the tank, the use of stainless-steel pipes means that less heating surface is required, which in turn reduces the flow resistance. Plastic pipes are used in the tank wall. **Table 7** shows an overview of integrated heaters.

Figure 14: Stainless steel heating pipes installed in the digester (inside) (left); installation of heating hoses in the digester wall (centre, right) (Fachagentur Nachwachsende Rohstoffe, 2013).

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Table 7: Tank heating integrated (Postel et al., 2008).

Type	Description	Range of application	Advantages	Disadvantages
Wall heater	Inside of tank	all types of fermenters, rather standing	Pipes accessible for work, e.g. for lime deposits, very good heat transfer	Flow resistance, Deposits, risk of tearing
	in the tank wall, heating hoses	In-situ concrete, not segment construction	Pipes protected	Temperature gradient in wall (stress cracks), reduced heat transfer
	Container exterior	Steel tank only	Pipes easily accessible	Reduced heat transmission
Floor heating	common floor heating cation lines	all standing fermenter	uniform temperature distribution	Low heat transfer, especially with sink layer formation
Heating in stirring unit	double wall pipe system	all digester types, rather horizontal	in the middle of the material for heating, good heat transfer	Damage to welds difficult to detect, less heat transfer during standstill

External heat exchanger: When using external heat exchangers, the substrate is heated outside the fermenter. This can be done in wet fermentation plants by heating in circulation or in the substrate feed (Persson et al., 2019). Heated percolate can temper the fermentation substrates in solid-state fermentation plants. Table 8 shows an overview of external heat exchangers. External heat exchangers can be used to avoid temperature fluctuations during substrate introduction.

As a rule, stainless steel is used as the material, and they are usually designed as spiral or double-tube heat exchangers. External heat exchangers are basically suitable for all types of fermenters; they are often used in plug-flow fermenters. Heat exchangers are well suited for thermophilic operation. External heat exchangers ensure good heat transfer, the entire biomass volume is reached by the heating. They can be easily cleaned and maintained and offer good temperature controllability (Fachagentur Nachwachsende Rohstoffe, 2013).

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Table 8: External tank heating *(Postel et al., 2008)*.

Type	Description	Range of application	Advantages	Disadvantages
Heating in circulation	Circulation of the fermenting liquid, e.g. via double-pipe or spiral heat exchanger	Wet fermentation	Tanks and walls are free of fixtures	increased electrical energy consumption due to pumping; deposits possible
Heating in the feed	Design of the inlet as a double-tube or spiral heat exchanger	Wet fermentation	Flexible handling, e.g. combination with hygienization	More elaborate tank insulation required; additional heating may be necessary; storage possible
Heated percolate	from temperature-controlled percolate storage for trickling over digestate in the solid's fermenter	Solid's fermentation	No or low-power heating devices required at the solid's fermenter	Carbonates in percolate liquid clog pumps, pipes and nozzles

1.3.3 Stirring systems

Functions of the stirring systems: Agitators must fulfil the following tasks in biogas plants (Kissel et al., 2014):

- Homogenization of the ingredients, this enables a uniform conversion of substances for the formation of biogas.
- Suspension of heavy inorganic substances that are introduced via the substrate.
- Reverse suspension: light, floating substances must be fed back into the bulk phase at varying levels. This serves to avoid the formation of swimming layers.
- Heat exchange in the container, uniform temperature distribution throughout the fermenter room for uniform material conversion.
- Complete circulation flow without demolition and formation of stagnant zones, for the discharge of biogas and even distribution of freshly introduced substrates.
- Uniform ground flow.
- Avoidance of blockages on the agitator

The design of the agitators is individual for each plant; there are no uniform concepts, as the ingredients and the process control vary depending on the plant. The technical requirements for the agitator can be subdivided according to the process. Biogas from wastewater plants can be classified as technically rather simple, since the solids content ranges between 3-6 % (Gomez, 2016). The organic content strongly predominates, the inorganic content is only 20

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%. The solids content in biogas plants is considerably higher, up to 22 % (Krieg et al., 2016). The technical effort to realise an effective agitation system is higher. In the following, some frequently used agitators are discussed in more detail.

Submersible mixer (propeller): This type of agitator is very common and has been used in biogas plants for a long time. Submersible propeller agitators are used in fully mixed fermenters as well as in digestate stores. In the fermenter, this type of agitator is often combined with other types of agitators (Mohammadrezaei et al., 2018). It is also possible that several agitators are operated in one tank.

The propeller and the electric motor form a unit that is completely immersed in the medium. Therefore, the entire agitator unit must be watertight and corrosion-resistant. Attached to a vertically arranged guide rod, the height of the agitator can be adjusted. The direction of action of the propeller can be changed by means of a crank, and the agitator unit can also sometimes be swivelled up and down by 30°. This makes them easily adjustable in position and is good against floating covers and sinking layers (Brehmer et al., 2016). The fermentation substrate cools the motor in the submersible mixer, in thermophilic fermenters make sure that the cooling capacity is sufficient (Bauer et al., 2019). Submersible mixers are counted among the fast-rotating agitators, they are equipped with small agitator blades (Ø 400 - 1000 mm) and have propeller speeds of up to 600 rpm. Due to the high speed, submersible mixers generate a large directional thrust, which is suitable for mixing low-viscosity fermentation substrates. The agitators have an output of between 0.25 and 35 kW. For fermenters between 24 and 28 m in diameter, common sizes are between 12 and 18 kW (Persson et al., 2019). With viscous media, such agitators only function to a limited extent; an effective mixing effect is only achieved with long agitation times. Viscous media damage or wear the agitator blades very quickly, which is why they should not be used in this application. Modified submersible mixers are available for this purpose; the agitator blades have a larger diameter (Ø >1000 mm) and lower speeds. There are also special designs of submersible mixers that are driven hydraulically and not electrically; these are operated by hydraulic units installed outside the fermenter (Bauer et al., 2019).

Figure 15: High-speed submersible mixer with cable guide on the suspension cable (Kissel et al., 2014).

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Submersible mixers are well suited for fermentation tanks with variable filling heights, the agitator blades must be completely submerged during operation to prevent imbalances. An advantage is that submersible mixers can also work at very low fill levels. Fast-running submersible mixers are better suited for stirring floating layers than slow-running submersible mixers, as they have a higher suction and pushing effect. The disadvantage is the relatively high energy input, and moisture can penetrate the motor.

Rod mixer (Figure 16 & 17): Like submersible mixers, rod mixers have long been used in biogas plants. They are used in fully mixed fermenters with a constant liquid level and are often combined with other agitators (Mohammadrezaei et al., 2018).

Figure 16: High speed rod mixer with smaller propeller (Kissel et al., 2014).

For low-viscosity media, the combination of submersible mixers and high-speed rod mixers has proven successful. For viscous fermentation substrate, paddle or long axis agitators are combined with slow-running large blade rod mixers. Propellers are attached to a drive shaft of the agitator, the number of which can vary. In the field of biogas production, rod mixers are driven by electric motors located outside the fermentation tank. Compared to submersible mixers, they require less maintenance and have a longer service life, which reduces costs. Like submersible mixers, rod mixers are fast agitators; the diameter of the agitator blades is relatively small (< 700 mm) and the speed of the propellers is high (up to 600 rpm). There are also other types of hand blenders with slower speeds (<100 rpm) and larger blades (Ø > 1200 mm). These slow-running stick mixers are used in the fermentation of substrates like corn or silage, which leads to an increase in dry matter content and viscosity (Kissel et al., 2014).

By changing the direction of rotation, the mode of action of the agitator can be changed from pushing to pulling. The shape of the agitator blade, the orientation of the agitator and the direction of rotation must be adjusted.

The rod mixers are mounted in the upper area of the fermenters, either guided through the fermenter wall or, in the case of a concrete ceiling, through the fermenter ceiling. To be able

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to counteract the formation of floating layers, the rod mixers are designed to swivel hydraulically, and their inclination can be varied (Bauer et al., 2019). Special forms of rod mixers exist; there is the application case of avoiding the formation of a sinking layer. For this purpose, rod mixers are mounted near the floor, they use a short agitator shaft and are often not swivelling. Compared to the submersible mixers, however, swivelling stick mixers are limited in their adjustment possibilities and cannot, for example, work tangentially to the container circumference.

Figure 17: Wall- and ceiling-mounted rod mixer with hydraulic (left) and mechanical (right) (Kissel et al., 2014).

Long-axis agitators (Figure 18): This type of agitator was specially developed for use in biogas plants. It is well suited for fermentation substrates with a high viscosity and for mixing viscous media. Due to their low speeds, they are not suitable for stirring floating blankets, at variable filling levels and for thin-bodied fermentation mixtures. Long-axis agitators are combined with slow-running large vane mixers or large vane submersible motors to achieve a good agitating result (Krieg et al., 2001).

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Figure 18: Long-axis agitator with ceiling and wall feed-through of the agitator shaft (Kissel et al., 2014).

Due to their large blade diameters (> 1.5 m), long axis agitators manage with low speeds (< 40 rpm) for effective mixing of the fermentation substrate. Long-axis agitators are mounted on the tank bottom as well as on the digester wall/ceiling. On tanks with a concrete ceiling, they are guided through the ceiling; on fermenters with an attached gas storage and weather protection foil, they are guided through the fermenter wall (Bauer et al., 2019). The drive unit of the agitator is mounted outside the fermenter/digester, which makes it easy to maintain. The speed of the agitators is adapted to the respective requirements with frequency converters. As soon as the medium is in motion, the agitator can switch to partial load operation, which means lower energy consumption (Bauer et al., 2019).

Paddle agitators (Figure 19): Like the long-axle agitators, this type of agitator does not originate from slurry technology but was developed for the fermentation of renewable raw materials and high dry matter content in the fermenter. Paddle agitators are available in vertical and horizontal versions.

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Figure 19: Suspended paddle agitator with drive unit (Kissel et al., 2014).

Horizontal paddle agitators: This type of agitator is mainly used in fully mixed fermentation tanks with a liquid level that is as constant as possible. They are rarely used in fermentation residue storage. In highly viscous media, they are combined with slow-running large vane rod mixers or large vane submersible motor agitators. Highly viscous media are present in fermenters that have a low proportion of liquid and a high proportion of solids. If the agitator shaft is at the same level as the substrate level, the direct effective radius is a paddle length.

Paddle agitators are usually very slow-running agitators, they run at a low speed of 1-20 rpm. The agitator shaft is equipped with large-dimension paddles, it is guided horizontally through the wall and mounted on a rod mounted in the middle of the fermenter. The drive motor is flanged to the agitator shaft outside the fermenter and is equipped with a torque stop and a frequency converter. This type of agitator is also easy to maintain in case of malfunctions. Horizontal paddle agitators are susceptible to corrosion processes, as creeping currents can occur in the agitator. To counteract this, care should be taken to ensure sufficient earthing of the agitator shaft.

Vertical paddle agitators: This type of agitator is also called an axial agitator and can be arranged centrically or eccentrically. Standing paddle agitators require a concrete ceiling to be technically attached. They are mounted on the ceiling and on the tank bottom. In most cases, the paddles are rigidly mounted on the agitator shaft and can be arranged in pairs or individually. They are suitable for use in fully mixed fermenters and for homogenising digestates in digestate stores. Vertical paddle agitators are often combined with other standing paddle agitators or other slow-running agitators.

Special forms: These types of agitators occur less frequently than the types listed above. They have been designed for special applications.

Reel agitators (Figure 20): This type of agitator has a long horizontal agitator shaft to which large agitator arms are attached. Compared to paddle agitators, the agitator shaft extends over the entire fermenter. With longer fermenters, an intermediate bearing is necessary to

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prevent sagging, which could otherwise lead to damage. Reel agitators have a very low speed of 1-5 rpm, they are operated without pause intervals. The drive units are located outside the fermenter (Weiland, 2010).

The reel agitator was developed for use in horizontal fermenters with high dry matter contents (>14 %) in the fermentation mixture. The aim of the agitation process is not to mix the fermentation substrate completely, but to prevent mixing in the horizontal (axial) direction. This causes a spatial differentiation of the decomposition phases. The agitator is not responsible for substrate transport; in the horizontal direction, the material is transported by the material feed (plug flow principle) in the tank. The advantage of the system is that the fermenter can be loaded more heavily and that short-circuit currents do not occur (Bauer et al., 2019).

A heater can be integrated into the agitator shaft. Heating water flows through the agitator shaft, which heats up the fermentation substrate. The failure of an agitator of this type should be avoided in any case, because the swelling of the fermentation substrate can damage the fermenter or the pipes. In the event of segregation of the fermentation substrate, the drive power is usually insufficient to restart the agitator. Warning alarms and an emergency power supply should therefore be installed to prevent damage (Bauer et al., 2019).

Meanwhile, reel agitators are available on the market for round pit fermenters. In these fermenters, they should intensively mix the entire fermenter contents. Difficult substrates can be fermented here, which have a strong tendency to segregate and push other agitators to their limits. Due to the high costs, they are only very rarely used in pit fermenters (Bauer et al., 2019).

Figure 20: Reel agitator in a horizontal fermenter with drive unit (Kissel et al., 2014).

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Central agitators (Figure 21): Like the reel agitator, it is used rather rarely, central agitators are used for mixing highly viscous fermentation suspensions. To be able to mix effectively, the fermenter geometry must have a certain ratio of fermenter diameter to fermenter height (0.8 to 1).

Central agitators are guided centrally through the concrete or stainless-steel ceiling of a high-level fermenter and mounted there. Two to three specially shaped agitator paddles are mounted on the agitator shaft in several levels. The agitator paddles are arranged in such a way that a downward flow is generated near the agitator and an upward flow at the edge of the fermenter. This allows viscous materials to be mixed effectively, and flow breakers are also used in this system to create further turbulence.

Central agitators are operated continuously to prevent the formation of floating layers. The drive unit is located outside the fermenter; for maintenance tasks on the agitator, it can simply be pulled out of the tank by means of a crane. The fermenter therefore does not need to be emptied.

Figure 21: Central agitator in fermentation tank (Kissel et al., 2014).

Hydraulic stirring (Figure 22): With hydraulic mixing, liquid is circulated in the fermenter, which should not be confused with the hydraulic drive of the mechanical agitators. Pumpable substrates are particularly suitable for hydraulic mixing. Hydraulic mixing has its origins in wastewater treatment, where mostly low-viscosity media occur. The disadvantage of this technology is that floating and sinking layers can hardly be separated. This technology is mostly used in small standing digesters (< 1500 m^3). In compact biogas plants, there is an increase in the hydraulic mixing system. These digesters have a working volume of about

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120 m^3 for good mixing of these types of tanks, hydraulic agitators are well suited (Bauer et al., 2019; Persson et al., 2019).

Thin liquid medium from the lower part of the fermenter is drawn off by circulation pumps and pumped from the top onto the substrate level. If pumping is sufficiently long or continuous, this can achieve complete mixing of the fermentation substrate.

Mechanical agitators have a considerably higher installation cost in direct comparison. The circulation pump is located outside the tank and is easy to maintain; there are no moving parts in the fermenter that are susceptible to failure (Weiland, 2010).

Figure 22: Centrifugal pump with cutter as circulation pump in the bypass line of a digester (Kissel et al., 2014).

Pneumatic mixing: With this agitator technology, the fermentation suspension is circulated by injecting biogas into the fermenter from below. Mixing occurs due to the displacement effect of the gas bubbles rising in the fermentation substrate. To achieve this, the hydrostatic pressure of the fermentation substrate must be overcome. Sufficiently powerful compressors and large enough gas pipes must be available. This technology is very rarely found in agricultural biogas plants. The process originated in wastewater treatment and is used in waste management plants with flowable substrates. The process is rather unsuitable for classical biogas plants, as the dry matter content in the fermentation mixture is too high. Pneumatic mixing is suitable for the exclusive fermentation of pure liquid manure, as the solids content is low enough. A resulting floating layer can hardly be stirred up, especially if substrates containing fibres with a tendency to form floating layers are used. The disadvantages of the process can be compensated for if they are combined with mechanical agitators. To increase the stirring performance, the gas is introduced into the fermenter via

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several nozzles. By recirculating the biogas, part of the introduced CO_2 is converted into methane, which increases the gas yield. There are no moving parts in the digester, the compressor is located outside the digester and is therefore easy to maintain (Bauer et al., 2019; Persson et al., 2019).

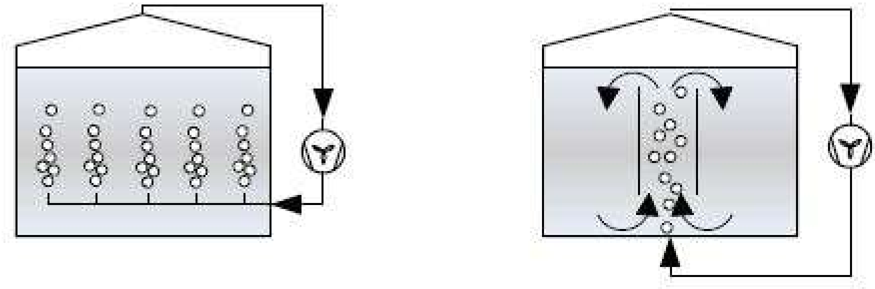

Figure 23: Basic principles for pneumatic mixing; gas spraying (left), gas lift (right) (Postel et al., 2008).

1.3.4 Conveying technology for liquid digestate

For substrate transport within a plant, sufficient mixing and the degree of comminution are the decisive factors. For this purpose, pipelines, fittings and pumps that transport liquids are considered. Gas pipes are dealt with in chapter 1.4.2 Conveying technology.

Fittings: Manually operated flat gate valves (Persson et al., 2019) are often used as fittings for shutting off containers. In smaller biogas plants, manually operated control levers are used to control the material flow. Larger systems are equipped with automatically actuated valves for control. These automatic control valves can be operated pneumatically or electrically. Pneumatic systems are preferred because they are significantly less expensive than electric valves. If the device is located far away from the compressor/compressed air system, electric fittings are used (Persson et al., 2019).

Pipes: Pipes made of steel or plastic are used to transport liquids. For pipes laid underground or in enclosed spaces, PE-HD pipe is mostly used, while stainless steel is used outdoors. Pipes laid underground must usually have a second casing to detect leaks. Plastic pipes are rarely used above ground because they tend to become brittle under UV radiation and require more elaborate support due to their lower mechanical strength. Steel pipes, on the other hand, tend to corrode when laid underground. Steel pipes are considerably more expensive than plastic pipes, which is particularly noticeable in the case of long stretches. Depending on the section of the system, the pipe may need to be thermally insulated, heated or frost-proofed. The pipelines are usually laid in such a way that the occurring operating cases can be controlled via easily accessible gate valves.

Pumps: In biogas plants, pumpable substrates are usually conveyed by pumps driven by electric motors (**Figure 24**). Together with the corresponding fittings, they can be controlled via process computers, which automate the process completely or partially. The pumps are

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often located centrally in one or more pump or control houses. Usually, one pump is used for as many pumping paths as possible. Substrate distributors are used to control what is pumped where by the pump.

Figure 24: Pumps in a biogas plant [WELTEC BIOPOWER GmbH] *(Fachagentur Nachwachsende Rohstoffe, 2013)*.

Even with good substrate preparation, the pumps can become blocked. They should therefore be easily accessible and there must be sufficient working space around them. The moving parts of a pump are wearing parts that need to be serviced or replaced regularly without the biogas plant having to go out of operation. It must therefore be possible to disconnect the pumps from the mains by means of a gate valve before carrying out maintenance work. As described in chapter 1.2.3, centrifugal or positive displacement pumps are mostly used, which are also used in slurry technology (DBFZ Deutsches Biomasseforschungszentrum., 2021).

Centrifugal pumps: Centrifugal pumps originate, as already mentioned, from slurry technology. Inside the pump, an impeller rotates in a stationary housing. The medium is accelerated by means of the impeller and the resulting increase in speed in the discharge connection of the centrifugal pump is converted into delivery head or delivery pressure (Bachmann, 2013). These pumps are also available in designs whose impeller is equipped with hardened cutting edges for substrate comminution. Common centrifugal pumps deliver a delivery pressure of up to 6 bar, which results in a delivery rate of from 2 m^3/min up to 6 m^3/min. The power consumption is strongly substrate-dependent and is e.g., 3 kW (for 2 m^3/min) and 15 kW (for 6 m^3/min). The pumps are generally suitable for a dry matter content of < 8 percent, making them suitable for low-viscosity substrates with a low straw content. Their robust design is simple and compact, they have a high flow rate and can be used flexibly. The disadvantage of this type of pump is that it is not self-priming, which means that the pumps have

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to be positioned below the level of the substrate to be sucked in. For this purpose, they can be placed in a shaft, for example. Due to the design, there is a high dependency between the delivery rate and the delivery pressure or delivery head. In terms of their design, centrifugal pumps are available as submersible pumps or as dry-installed pumps (Fachagentur Nachwachsende Rohstoffe, 2013).

Eccentric screw pumps: Eccentric screw pumps (see figure 7) are self-priming, valveless, eccentrically rotating positive displacement pumps. The pumping principle is based on a corkscrew-shaped rotor that runs oscillating in a stator made of elastic material. The two components are matched to each other, forming a delivery chamber through which the medium is transported. The design of the rotor and stator means that there is hardly any pulsation or shear forces. The medium can therefore be moved continuously. The flow rate can be regulated via the speed, and in combination with a frequency converter, the flow rate can be precisely controlled. The pumps reach a delivery pressure of up to 48 bar and enable a delivery rate from 0.055 m^3/min up to 8 m^3/min. They draw a power of 7.5 kW at 0.5 m^3/min and 55 kW at 4 m^3/min. The power consumption is strongly dependent on the substrate being pumped. The advantage of these pumps is that they are self-priming, have a simple and robust design, are highly meterable and the direction of rotation can be reversed. They have a stable delivery at fluctuating pressures. Dry-running protection can be integrated. The disadvantages are that they have a lower delivery rate than centrifugal pumps, they are sensitive to dry running and they also react sensitively to impurities such as stones or metal particles. The pumps are very durable, easy to maintain and require only a short maintenance period (Fachagentur Nachwachsende Rohstoffe, 2013).

Rotary lobe pumps: Rotary lobe pumps (see Figure 23) are self-priming, valveless positive displacement pumps. They have two counter-rotating two- to six-bladed rotary pistons. The rotation of the pair of pistons creates a vacuum on the suction side. The pistons do not touch each other. The vacuum draws the liquid into the pump chamber and, by further rotating the pistons, transports it into the pressure area. Rotary lobe pumps can generate a delivery pressure of up to 12 bar. The flow rate is between 0.1 m^3/min and 16 m^3/min. The power consumption is between 2 and 55 kW. They are suitable for viscous, pumpable substrates. They have a simple and robust design, are self-priming up to 10 m water column and suitable for substrate dosing. They convey larger foreign and fibrous materials than eccentric screw pumps, are insensitive to dry running, require little space and are easy to maintain (Fachagentur Nachwachsende Rohstoffe, 2013).

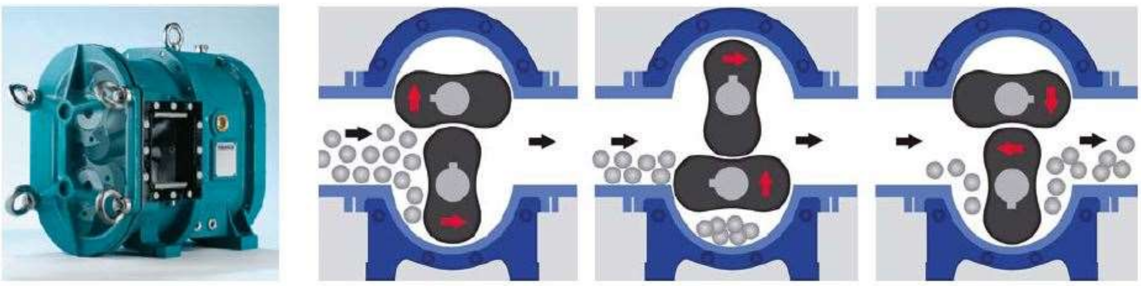

Figure 25: Rotary lobe pump (left), rotary lobe pump principle (right) [Börger GmbH (left), Hugo Vogelsang Maschinenbau GmbH] (Fachagentur Nachwachsende Rohstoffe, 2013).

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1.4 Utilisation of biogas

1.4.1 Intermediate storage in the gas storage facility

Flexibilization: Through the digesters, biogas is usually produced constantly and through feeding, gas production should be kept at a constant level in the long term. To compensate for small differences between gas production and gas utilisation during continuous electricity generation, biogas plants have gas storage facilities. In the case of demand-oriented feed-in (flexible operation), biogas storage has an additional significance. Discontinuous utilisation of the biogas is made possible by regularly emptying and filling the gas storage tanks. In a flexible mode of operation, the storage system holds the biogas to be able to run at increased output during attractive hours. Fluctuations in biogas production can be compensated for in flexible operation by adjusting the runtime duration on the following day. (Fachagentur Nachwachsende Rohstoffe, 2018; www.biogas-netzeinspeisung.at, accessed 07.10.2021).

The dimensioning of the gas storage depends on the operating strategy of the biogas plant. With constant gas output, the necessary size of the gas storage capacity increases with the degree of flexibilization. With increasing plant output, the necessary storage capacity grows. On the one hand, the storage size determines the maximum idle time and, on the other hand, the maximum running time of the CHP unit. The capacity of the biogas storage should generally be more than 12 hours, because the peak price periods that occur in the typical daily rhythm have approximately this time interval (daily flexibility) (Fachagentur Nachwachsende Rohstoffe, 2018). With a larger storage volume, the costs of interval operation for the CHP can be reduced because it has to be started less frequently (www.biogas-netzeinspeisung.at, accessed 07.10.2021). In principle, the construction costs for flexible plants are higher than for non-flexible ones. Whether this makes economic sense depends mainly on whether demand-responsive feed-in is a prerequisite for government subsidies for electricity remuneration.

Gas storage: Gas storage tanks must be pressure-resistant, gas-tight, resistant to media, UV, temperature, and weather. (Weiland, 2010). Before commissioning, they must be checked for tightness. Gas storage tanks are equipped with mechanical or hydraulic over pressure and under pressure safety devices to ensure that the internal pressure remains within the prescribed limits.

Container-bound storage systems: This refers to a round container that is covered with a gas-tight dome-shaped roof. A general advantage of container-based storage systems is the space saving compared to external gas storage systems, as the containers are already available. If the fermenter or the secondary fermenter itself is used as a gas storage, foil roofs are usually used. In the case of a concrete cover and a constant gas volume, the gas space cover merely provides a gas-tight seal of the fermenter against the environment. With variable gas volumes, the gas storage serves to compensate for fluctuating gas production. For

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the subsequent section, the gas storage tank provides a constant gas volume flow and gas pressure.

The tank covers can be designed as a concrete cover, made of stainless steel, from a **single-membrane storage tank** or **double-membrane storage tank**. Only the last two tank covers mentioned are suitable for flexibilization. A comparison of the different covers can be found in Table 9. When using foils, these are attached gas-tight to the upper edge of the container. A support frame is built onto the tank on which the foil can rest when the gas storage tank is completely empty. Roofs consisting of only one foil must be expandable and must be able to expand depending on the filling level of the gas storage tank. Supporting air roofs (see **figure 26**) or double membrane storage tanks consist of two, non-expandable foils.

Figure 26: Biogas plants with load-bearing roofs (AEV Energy GmbH)

The upper film is a weather protection film that is applied over the actual storage film. The weather protection foil serves to protect against environmental influences (especially wind gusts) and to absorb acting loads. Air is blown into the space between the two foils. The supporting air blown into the gap ensures the stability of the outer film and causes a relatively constant pressure on the inner membrane. The upper weather protection foil is always in a taut, stretched state, while the storage foil adjusts variably to the amount of biogas to be stored. With this system, the gas pressure is kept as stable as possible (Weiland, 2010).

In principle, the container cover is independent of the type of material used in the fermenter. Container-bound gas storage systems are always pressure less or low-pressure storage systems; the pressure range is between 0 and 5 mbar for simple foil storage systems and 0 - 50 mbar for double membrane storage systems. The sealing of the foil roofs is achieved by means of a so-called Seeger seal or with clamping rails (Persson et al., 2019).

Gas storage membranes have a certain diffusion rate of 1-5 ‰, concrete and steel covers are emission-free. Storage foils are mostly made of PE or fabric-reinforced PVC. Some odour emission is released by diffusion, which does not require any further measures during normal operation. The odour emission is reduced in double-membrane storage tanks.

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Table 9: Overview of container covers and container-bound gas storage tanks (Postel et al., 2008).

Type	Description	Application area	Advantages	Disadvantages
Concrete ceiling	attached steel concrete plate	only for concrete tanks stable	stable, can be walked on, partly driven over, suitable for agitators	constant gas volume not suitable as "lung function
enamelled steel or Stainless steel	Steel construction segments, foil as inner membrane	Concrete tanks, steel tanks	lighter than concrete, better to install	Gas volume somewhat larger, but still inflexible
Single-membrane storage tank (single shell)	foil cover made of a single membrane - mast supported	all standing tanks widespread in older and small plants	larger storage volume low cost possibility of optical process control	constant gas volume, susceptible to weather, wind and snow Foils can tear under negative pressure
Double-membrane storage tank (double-shell)	inner foil as gas-tight membrane, outer foil as protective cover - mast supported - Supporting air roof	for all standing containers, is standard	larger storage volume, more weather-resistant than single-skin possibility of optical process control permanently low pre-pressure supplied	More expensive due to increased film requirements Foils can tear under negative pressure Energy requirement for supporting air

External storage systems: The gas storage tank is spatially decoupled from the fermenter/digestate storage. They are available as low-pressure, medium-pressure and high-pressure storage tanks. The advantage is that less gas is lost when the fermenter is opened. The disadvantage is the increased space requirement (Khanet al., 2017).

External low-pressure storage tanks can be designed in the form of foil cushions or supporting air storage tanks on concrete foundations. To protect them from the weather, they can be covered with an additional foil or housed in a building. The advantage of this system is the low cost compared to other systems, and the usable volume of foil cushions is relatively large. The disadvantage is the short life of the foil if it is exposed to the weather. The basic principle is the same as for internal double-membrane storage. An outer foil is supported by supporting air, which protects the movable storage membrane inside (Persson et al., 2019). An overview of the types of storage tanks in the low-pressure range is given in **Table 10**; an example of an external double-diaphragm storage tank is shown in **Figure 27**.

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Table 10: Overview of external gas accumulators in the pressure less and low-pressure range (Postel et al., 2008).

Type	Description	Application area	Advantages	Disadvantages
Foil pillow feeder	in fixed old buildings (silo, barn) in new building, e.g., lightweight hall on concrete ceiling of a digester exposed or under roofing	Farms that have buildings available Special cases, e.g., if digester is underground tanks with concrete ceiling smaller storage tanks	inexpensive with existing building easily accessible space-saving easily accessible	Volume (shape/ quantity) limited if necessary additional space required relatively small volume exposed to weather and sun
Complete system	Double membrane accumulator	Accumulator can be positioned on terrain	flexible and modular installation	additional space requirement

Figure 27: Example of freestanding double diaphragm accumulator. (AEV Energy GmbH)

1.4.2 Conveying technology for biogas

In normal operation, only biogas is transported in the gas-carrying system. This must comply with special safety regulations, as the escape of gas or the entry of air can form explosive mixtures. In case of malfunctions, the liquid level in the fermenter may rise above the opening edge of the gas outlet, thus digestate or foam may enter the gas line. Gas pipes must be inspected and maintained regularly; corrosion increases the risk of gas leaks and explosions. Raw biogas also contains water vapour, hydrogen sulphide, hydrogen and ammonia, which

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are reactive gases that can attack metallic pipelines. Water collected in the condensate separator can corrode the pipes. The pipelines must therefore be resistant to media and corrosion; they are made of stainless steel, polyethylene (PE-HD) or PVC-U. In general, stainless-steel pipelines are often used above ground; plastic pipelines must be protected from mechanical and thermal damage and need more supports than stainless steel pipes when laid above ground. The connections are flanged, welded, glued or screwed. All fittings and pipelines must be protected against frost. The pipes are always laid with a gradient, this enables targeted condensate collection. It must be possible to drain condensate from all gas pipes, all fittings must be easily accessible and easy to maintain. When laying pipes in the ground, ensure good compaction, the laying must be stress-free, if necessary, compensators or U-bends must be planned.

Figure 28: *two tanks with piping and pressure-relief device (AEV Energy GmbH)*

1.4.3 Gas treatment

In addition to methane (CH_4) and carbon dioxide (CO_2), general raw biogas also contains considerable amounts of hydrogen sulphide (H_2S) and ammonia. The combination of hydrogen sulphide and the water vapour contained in the biogas results in acid formation (Fachagentur Nachwachsende Rohstoffe, 2013), which leads to corrosion. Most modern CHP units are equipped with oxidation catalysts; sulphur atoms can occupy the active centres of the catalysts even in moderate concentrations and thus render the catalysts ineffective. Water vapour in larger quantities is adsorbed by the activated carbon and inhibits the absorption of sulphur. In the case of biogas plants, desulphurisation and drying of the biogas is usually carried out. The manufacturers of CHP units set minimum requirements for the quality of the fuel gases used; these should be complied with in order to be able to operate the CHP units effectively and to protect them from damage (Fachagentur Nachwachsende Rohstoffe, 2018).

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Desulphurisation: Possible processes (for comparison see **Table 11**) can be divided into biological, chemical and physical separation processes. In addition to the gas composition, the flow rate is a decisive parameter for the desulphurisation equipment. The flow rate can fluctuate; it is increased when fresh substrate is fed in and during operation of the agitators. Power peaks of the flow rate can therefore occur by more than 50 % above the average value. Therefore, the plants must be appropriately dimensioned to be able to absorb the peaks. Several processes can be switched in succession.

Biological desulphurisation in the fermenter: Biological desulphurisation is often carried out directly in the fermenter. By means of the bacterium *Sulfobacter oxydans*, hydrogen sulphide is converted with oxygen into elemental sulphur (Ramos et al., 2013).

$$2\ H_2S + O_2 \longrightarrow 2\ S + 2\ H_2O$$

The sulphur accumulates on the surface of the tanks and eventually falls back into the substrate. The elemental sulphur can therefore be discharged from the reactor via the fermentation residue. The bacteria are already present and do not have to be added separately. The oxygen is introduced into the fermenter by injecting defined amounts of air (max. 5 % of the biogas formed in the same period). This process is very cost-effective, no additional chemicals are required. The technology is low-maintenance and low-failure. Sulphur can then be discharged as fertiliser. The introduction of oxygen may impair the process and methane oxidation is possible. It is difficult to react to different gas production rates. Another disadvantage of this process is the possible formation of sulphuric acid. Bacteria can develop (in the presence of atmospheric oxygen) that oxidise the resulting hydrogen sulphide to sulphuric acid. This leads to corrosion damage on surfaces susceptible to this (concrete and metal materials). The use of biological desulphurisation has proven itself in technology; its effectiveness depends on the sulphur content. It is hardly possible to reduce the hydrogen sulphide concentration to the level required for combustion in a CHP with oxidation catalyst, but the process improves the gas quality in a simple way to such an extent that downstream methods can work more economically. It is therefore mostly used in combination with other processes.

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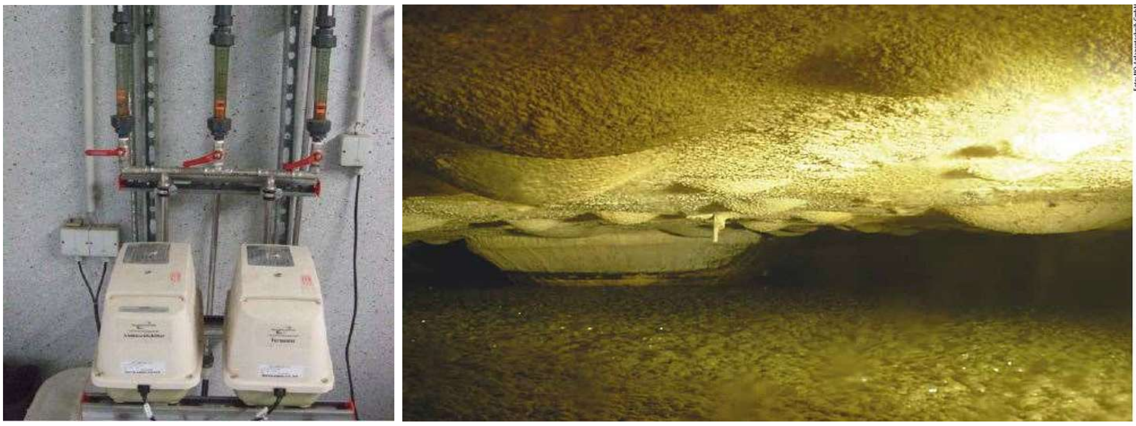

Figure 29: Left figure: Gas control for air injection (Fachagentur Nachwachsende Rohstoffe, 2013). Right figure: Sulphur in the fermenter (Biomin, accessed 18.10.2021).

Biological desulphurisation in external reactors - trickling filter process

Biological desulphurisation is also possible outside the plant. Separate containers with biological desulphurisation columns can be used for this. In the droplet process, hydrogen sulphide is absorbed with the help of a scrubbing medium. Degradation rates of 99 % can be achieved, which can lead to a residual gas concentration of less than 50 ppm sulphur. The technology is available for all biogas plant dimensions and is basically suitable for all biogas plants. This process is rather unsuitable for feeding into the natural gas grid due to the high air input. The advantage is that the air input takes place outside the fermenter, so the process is not impaired by the oxygen input. Targeted automatic regulation of hydrogen sulphide decomposition (through nutrient, air supply and temperature management) is possible. No use of chemicals is necessary. The disadvantage is that an additional, costly unit is needed and the high air input in the biogas. Another disadvantage is that maintenance is very expensive (Okoro and Sun, 2019).

Figure 30: External biological desulphurisation, on the left bio-trickling bed reactor, on the right bio-moisture reactor [S&H GmbH & Co. Umweltengineering KG] *(Fachagentur Nachwachsende Rohstoffe, 2013)*.

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Sulphide precipitation: Sulphide precipitation is a chemical process which, like biological processes, is used for coarse desulphurisation. With sulphide precipitation, hydrogen sulphide values between 100 and 150 ppm are achieved. By adding ferric chloride or ferric hydroxide, the sulphur is chemically bound in the substrate, thus preventing its release as hydrogen sulphide.

$Fe^{2+} + S^{2-} \longrightarrow FeS$

$2\ Fe^{3+} + 3\ S^{2-} \longrightarrow 2\ FeS + S$

The iron salts are dosed via the preliminary pit or the solids dosing unit or directly into the fermenter. The hydrogen sulphide formed is still bound in the liquid phase of the fermenter as sparingly soluble iron sulphide. The iron sulphide remains as a solid in the digestate and can be discharged from the system with the solids discharge. The exact dosing quantity depends on the respective system; it is usually between 100 - 220 $g_{iron}/t_{substrate}$. The dosing quantity is strongly dependent on the substrate used. The binding of H_2S directly in the fermenter can have a positive effect on the microbiological processes taking place (Fachagentur Nachwachsende Rohstoffe, 2013). Sulphide precipitation is also often used in combination with biological desulphurisation.

Adsorption on activated carbon: For the upgrading of biogas to biomethane and its injection into the natural gas grid and for the operation of CHPs with oxidation catalysts, very low H_2S concentrations are necessary in the biogas. To achieve this, fine desulphurisation is necessary. Activated carbon filters are very often used for this purpose. This process uses adsorption on activated carbon based on the catalytic oxidation of the hydrogen sulphide on the activated carbon surface. The hydrogen sulphide is adsorptively bound in the pore system and then catalytically oxidised. Impregnation or doping of the activated carbon to improve the reaction rates and the loading capacities is possible. Potassium iodide and potassium carbonate are used as impregnating agents. During operation, the activated carbon filters are loaded once with carbon. This is used until the loading limit is reached. When the activated carbon loses its effect can be determined by measuring the hydrogen sulphide content after desulphurisation. The loaded activated carbon is then either regenerated or disposed of in a waste incineration plant, for example. In order to increase the service life of activated carbon filters, a combination with coarse desulphurisation is recommended, which makes the process more economical (Okoro and Sun, 2019).

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Figure 31: Activated carbon filter biogas with base and bypasses (BioBG, accessed 25.10.2021).

Table 11: Process overview desulphurisation process (Fachagentur Nachwachsende Rohstoffe, 2013).

Procedure	Energy demand		Operating supplies		Air input	Purity in ppm	Problems
	el.	them.	Consumption	Disposal			
Biological desulphurisation in the Fermenter	++	0	++	++	yes	50-2.000	Inaccurate process control
External biological desulphurisation	-	0	+	+	yes	50-100	Inaccurate process control
Bioscrubber	-	0	-	+	no	50-100	High procedural effort
Sulphide precipitation	0	0	--	0	no	50-500	Inertial process
Activated carbon	0	0	--	-	yes	<5	High disposal volumes

++ particularly advantageous, + advantageous, 0 neutral, - disadvantageous, -- particularly disadvantageous

Drying: To optimise combustion in CHP units and meet the requirements of subsequent purification stages, water vapour must be removed from the raw gas. The amount of water vapour in the biogas depends on the gas temperature. In the fermenter, the relative humidity is close to 100 % and the biogas is saturated with water vapour. The processes used to dry the biogas are condensation drying, adsorption drying and absorption drying (Okoro and Sun, 2019).

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Condensation drying: To separate the condensate, cooling takes place within the gas pipe. The operating principle of this process is based on cooling the biogas below the dew point. In small systems, the cooling of the gas as it passes through buried pipes can already remove sufficient water. For this purpose, the gas pipes are laid at an incline, and at the lowest point of the pipe the condensate is collected at a condensate separator. For the gas to cool down, it must have a certain residence time in the pipeline. In addition to the water vapour, other undesirable constituents such as water-soluble gases and aerosols are removed from the biogas. The separator must be emptied regularly, so it must be easily accessible for maintenance work. In larger plants, the flow velocity is often too high for all the water to be separated in this way. In this case, additional gas cooling systems are used to reduce the dew point. Dew points of 3 -5 °C are thus possible. The efficiency can be further increased by prior compression (Fachagentur Nachwachsende Rohstoffe, 2013). After gas cooling, the absolute humidity is lower; the relative humidity is adjusted by reheating the gas. This value is important for the operation of activated carbon filters and combustion engines. Condensation drying is usually sufficient for the needs of a biogas plant.

Adsorption drying: The process is based on zeolites, silica gels or aluminium oxide and allows dew points of up to -90 °C. The adsorbers are mounted in a fixed bed and are operated alternately at an ambient pressure of 6 to 10 bar. The process is suitable for small to medium volume flows but is rather untypical for biogas plants.

1.4.4 Combined heat and power plant

With a combined heat and power unit (CHP), electricity and heat can be generated simultaneously. The heat is produced as a co-product and is used to provide the process energy and for other external purposes (ASUE, 2014). Combined heat and power plants with combustion engines coupled to a generator are used almost exclusively. The engines run at a constant speed, which allows the generator to provide electrical energy whose frequency is compatible with the grid frequency. Usually, ignition jet and gas Otto engines are used, but alternatively micro gas turbines, Stirling engines or fuel cells can be used.

A CHP module (see **Figure 32**) consists of the following components: an internal combustion engine, a generator, a heat exchanger system, cooling water and lubricating oil circuits, hydraulic equipment for heat distribution and electrical switching and control equipment (Weiland, 2010).

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Figure 32: Biogas CHP, complete module in compact design with emergency flare (Fachagentur Nachwachsende Rohstoffe, 2013).

Gas-Otto engines: These are engines specially developed for gas operation, which work according to the Otto principle. To minimise nitrogen oxide emissions, gas Otto engines in biogas plants are operated as lean-burn engines. This leads to a reduction in performance and less fuel is converted in the engine. Gas Otto engines rely on a minimum methane content of 45 %; at lower concentrations, the engines shut down. Gas Otto engines are mainly used in the higher power range. They achieve mechanical efficiencies of 40-42 % at full load (at a rated power of > 300 kW). The overall efficiency is high at up to 90 %, and the operating time is about 80,000 - 96,000 operating hours (Persson et al., 2019). The electrical output is up to > 1 MW, rarely less than 100 kW. Engines of this type are basically suitable for all biogas plants, but economically they are more suitable for larger plants. They are specially designed for gas utilisation; emission limits are largely complied with. They require little maintenance and the overall efficiency is higher than that of ignition jet engines. The disadvantages are the higher investment costs compared to ignition jet engines and a lower electrical efficiency than ignition jet engines in the lower power range (ASUE, 2014).

Dual Fuel Engines: Engines of this type work according to the diesel principle. The biogas is added to the combustion air via a gas mixer. Ignition oil is fed into the combustion chamber via an injection system and ignited together with the gas mixture. The proportion of ignition

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oil in the fuel supplied is approx. 2 to 5 %. Ignition jet engines are operated with a high air surplus, they are mainly used in the small power range up to 350 kW. The operating time is about 35,000 operating hours, they achieve a mechanical efficiency of 30 to 44 %. The efficiency increases with increasing plant size.

In principle, these engines can be used for all biogas plants, but for economic reasons they tend to be used in smaller plants. They are less expensive than gas Otto engines and have a higher electrical efficiency in the lower power range compared to the gas Otto engines. Due to the relatively small amounts of ignition oil used, cooling is rather low and there is a risk of coking of the injection nozzles. This leads to increased exhaust gas loads and thus to shorter maintenance intervals. The overall efficiency is lower than with gas Otto engines, and an additional fuel (ignition oil) must be used. In the event of a biogas supply failure, it is possible to switch to a substitute fuel (diesel), for example to ensure heating of the fermenter (Weiland, 2010).

Generators: The generator is the interface between the mechanical movement and the flow of electricity. The combustion engine generates movement, and this is converted into electricity by the generator. Synchronous generators are mostly used in CHP units, but asynchronous generators are also used in some cases. The generators are usually coupled directly to the shaft of the combustion engine. Digital voltage regulators are a component of the generators, which enables them to ride through voltage dips in the grid and support the dynamic grid support.

Heat extraction: Heat is generated during electricity production in a CHP unit, and heat exchangers can be used to utilise this heat. The largest amount of heat is produced in the cooling water circuit of the combustion engine. Due to its temperature level, this can be used to provide heating or process energy. Plate heat exchangers are usually used to extract the heat from the cooling water. The heat thus extracted is fed to the individual heating circuits via a distributor. The exhaust gases of combustion engines contain a lot of energy; their temperature level is approx. 460 to 550 °C. The heat is usually extracted from the cooling water by means of plate heat exchangers. Exhaust gas heat exchangers made of stainless steel are mostly used for heat recovery; steam at various pressure levels, hot water and thermal oil are used as heat transfer media (Fachagentur Nachwachsende Rohstoffe, 2013).

The heat produced can be used internally in the plant. The heat demand depends on the season; in winter it is high, in summer excess heat has to be dissipated by emergency coolers. The heat required for fermenter heating is about 20 % to 40 % of the total heat produced. In addition, plant rooms and living quarters can be heated. In addition to internal heat sinks, the heat can be consumed externally. Particularly due to the expensive purchase of renewable natural resources, an economical use of the heat produced is recommended and is often also remunerated with bonuses. Heat consumers can be nearby commercial or communal facilities, for example horticulture farms, fish farms, wood drying, swimming pools and residential buildings. For heat transport, sufficient thermal insulation is recommended to potentially save heat (Effenberger et al., 2008).

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1.4.5 Biomethane injection

Biogas can be upgraded to biomethane (equivalent to natural gas) and fed into the natural gas grid. To do this, the non-combustible gases must first be removed from the biogas, and then the calorific value and pressure must be adjusted to the tolerances of the natural gas network. The entirety of these processes is called biomethane upgrading. Depending on the process and the plant constellation, this is either downstream of conventional gas treatment or does not require it. Biomethane feed-in is often operated in parallel with combined heat and power generation. An existing natural gas network in the vicinity of the biogas plant is advantageous for economical use.

Carbon dioxide separation: The processing step of carbon dioxide separation is required for the subsequent feeding of the product gas into the grid. By increasing the methane content, an adaptation to the required combustion properties of the product gas is possible.

Pressure Swing Adsorption (Figure 33): Pressure swing adsorption is an adsorptive biogas upgrading process. Adsorption is the accumulation of gas particles (here CO_2) on the surface of solids (adsorbents) (Fachagentur Nachwachsende Rohstoffe, 2018). Activated carbon, zeolites or carbon molecular sieves are used as adsorbents. In addition to CO_2, other gas components such as water or hydrogen sulphide, nitrogen and oxygen are also retained in this process. With the process of physical adsorption and desorption alternating by pressure change, CH_4 yields of 97 percent are achieved.

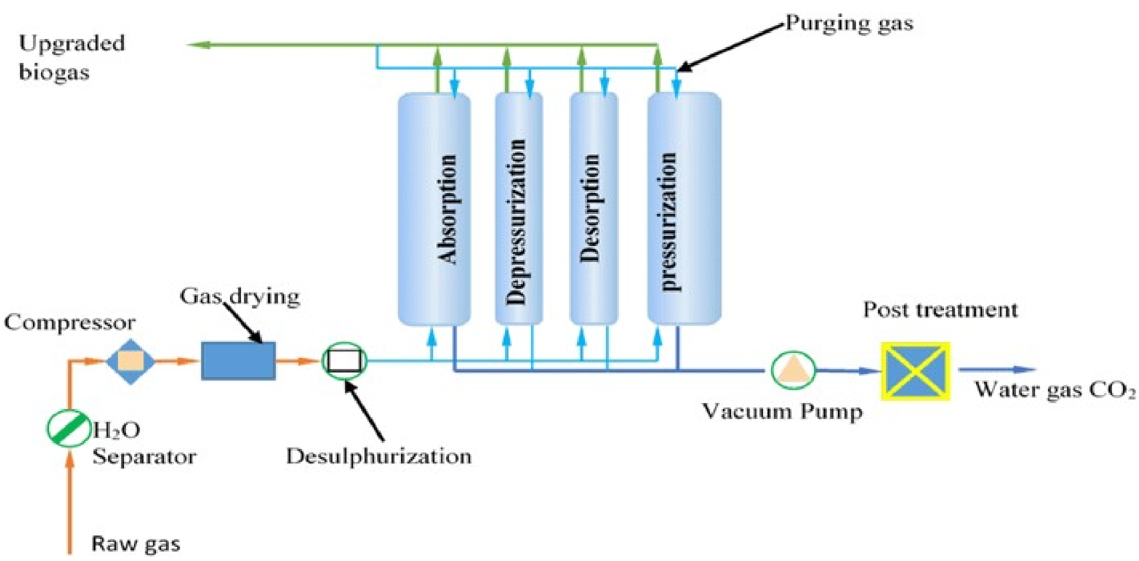

Figure 33: Pressure Swing Adsorption Technology for CO_2 Removal (Ogunlude, 2019).

First, a pressure increase of approx. 4 to 7 bar takes place in this process. This is followed by water separation and desulphurisation. The gas is then fed into an adsorption column containing a molecular sieve. The carbon dioxide is retained on the molecular sieve, and the

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methane passes through the column almost completely. A small amount of methane is also retained and discharged with the CO_2 (off gas). The adsorbed gas components are desorbed by lowering the pressure. A first partial flow of the desorbed gas, which still contains CH_4, is fed into another column that is still unloaded. The methane flows through the column as completely as possible, whereby other components are retained again. A vacuum is applied to the first column, resulting in complete desorption of the column (Alonso-Vicarioet al., 2010). The methane yield can be further increased by recirculation and additional purging cycles. The disadvantages of this process are the relatively high-power consumption, the low controllability of the system and the high methane loss in the exhaust air stream (approx. 1 to 5 %). Before using the PSA process, desulphurisation and drying of the raw gas is necessary. The heat requirement can be classified as low and no additional process chemicals are used (Alonso-Vicarioet al., 2010).

Pressure washing: This process is an absorptive biogas upgrading process. Absorption is the dissolving of gases in liquids (absorbents). In pressurised water scrubbing, water is used as the absorbent. The operating principle of this process is based on the different solubility of methane and carbon dioxide and other gases (Xiao et al., 2014).

First, the raw gas is brought to a pressure level of approx. 7 to 10 bars by means of compression. It then enters an absorption column from below and flows through it. Water flows through the column from top to bottom in counterflow. Water, hydrogen sulphide, carbon dioxide, ammonia and any dust and microorganisms contained in the raw gas dissolve in the column (Fachagentur Nachwachsende Rohstoffe, 2013). The raw gas is then saturated with water, leaves the column at the top and still must be dried. Methane is still bound in the absorbent; to remove this, it is partially depressurised in a flash column. The desorbed gas leaves the flash column at the upper end and is fed into the raw gas stream. In a desorption column, the water is expanded and brought to ambient pressure. The water thus regenerated can now be used again as a scrubbing agent for absorption. The dissolved exhaust gas leaves the desorption column at the upper end and usually must be fed into an exhaust gas after treatment system (Xiao et al., 2014).

A methane content of up to 98 per cent can be achieved. It is not necessary to carry out desulphurisation or drying before the pressurised water scrubbing. Another advantage of this process is the flexible adjustment to the gas volume; depending on the CO_2 content of the raw gas, pressure, temperature, and throughput can be regulated. Automatic and continuous operation is possible, and maintenance can be classified as relatively easy. A disadvantage is the relatively high-power consumption and the high methane slip of approx. 1 % (Xiao et al., 2014).

Amine scrubbing: Amine scrubbing is a chemical absorption process in which biogas is brought into contact with a scrubbing liquid. Monoethanolamine or diethanolamine (ethanolamine-water mixtures) are used as washing media for CO_2 separation. If H_2S is also to be removed, methyl diethanolamine or triethanolamine are used as washing media.

In contrast to physical washing processes, absorption can take place almost without pressure. There are also manufacturers who compress the gas to up to 4 bars before it enters the column. Co-absorption of H_2S takes place in the scrubber, so fine desulphurisation takes

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place simultaneously. Oxygen in the raw gas has a negative effect on the process; this can lead to undesired oxidation of the absorbent. Regeneration of the loaded absorbent takes place in the desorber with the addition of heat at 110 to 160 °C (Vo et al., 2018).

The process is suitable for rather smaller volume flows. The regeneration stage by steam has a high thermal energy requirement, which speaks against this process. Another disadvantage is the high detergent requirement. An advantage is the very high product gas quality > 99 % (prerequisite for this is low N_2 and O_2 concentrations in the raw gas) and the low methane slip of < 0.1 % (Vo et al., 2018).

Membrane process: A relatively new process, but one that is already in use. In terms of process technology, membrane processes effect the separation of gases through the different diffusion velocities of the gas molecules of different sizes (Chen et al., 2015).

Because CO_2 molecules are smaller than methane molecules, they can pass through the micropores of the membrane more quickly. Methane accumulates on the high-pressure side of the membrane, while unwanted substances such as water vapour, ammonia, hydrogen sulphide and CO_2 pass through the molecular sieve. The process requires prior desulphurisation and drying and entails a very high-power requirement with low heat demand. The achievable methane content is about 96 %. No additional chemicals are used, and the methane slip is relatively high (Chen et al., 2015).

Cryogenic process: This process is based on gas liquefaction by rectification. Liquid CO_2 is produced, which is filtered out during a low-temperature separation. It is a very demanding process that requires prior desulphurisation and drying of the biogas.

The cryogenic process is only used in pilot plants, has a very high electricity demand, but no chemicals are used, and the methane slip is minimal (Tan et al., 2017).

Processing to natural gas quality: The final processing stage of gas upgrading to natural gas quality can be divided into three process steps: odorization, calorific value adjustment and pressure adjustment.

Odorization: In this process, odorous substances are added to the biogas. Mercaptan or tetrahydrothiophene are mainly used. These strong-smelling substances serve to enable the human sense of smell to detect leaks. For technical and ecological reasons, sulphur-free odorants are increasingly being used (Fachagentur Nachwachsende Rohstoffe, 2013).

Calorific value adjustment: For injection, the biogas must have the same combustion properties as the adjacent natural gas. A measure of this is the calorific value, the relative density and the Wobbe index (Fachagentur Nachwachsende Rohstoffe, 2013). The actual values may lie within the permissible fluctuation range, the relative density may be temporarily exceeded, and the Wobbe index may be undershot. If the calorific value in the biogas is too high, the required parameters can be achieved by adding air. If the calorific value in the biogas is too low, regulation/adjustment is carried out by adding a propane-butane mixture (Fachagentur Nachwachsende Rohstoffe, 2013).

Pressure adjustment: To feed in different network levels, a slightly higher pressure is required than the existing network pressure. For low-pressure networks this is < 0.1 bar, for

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medium-pressure networks between 0.1 - 1 bar, for high-pressure networks from 1 bar and for ultra-high-pressure networks from 16 bar. Screw and piston compressors are used to compress biogas (Fachagentur Nachwachsende Rohstoffe, 2013).

1.4.6 Thermal utilisation of biogas

Depending on regional regulations, alternative gas consumption facilities (as an alternative to the CHP) are usually prescribed for biogas plants. These serve to avoid gas emissions in the event of a CHP failure. As a rule, this is realised by means of emergency gas flares, which are sometimes already integrated into the CHP unit. Alternatively, gas burners can also be used if the heat is to be utilised.

Gas burner: In practice, the combustion of biogas for the exclusive generation of heat is usually only used in combination with cogeneration. This makes sense, for example, if the plant has a gas heating system anyway, which can then also use biogas as an alternative. Units with atmospheric burners and gas blower burners can be used for this purpose. Atmospheric burners fire boilers, condensing boilers or instantaneous water heaters in the output range from 5 to 30 kW. The heat generators are usually integrated into a system with buffer storage to which the heat consumers are connected (Chandra et al., 1991). Larger output ranges are covered by forced draught burners.

Direct biogas combustion is used almost exclusively in heating plants that belong to the industrial anaerobic wastewater treatment and sewage gas utilisation.

Emergency flare (Figure 34): Gas emergency flares are widespread safety devices on biogas plants; here, the focus is on safety; heat utilisation does not take place. A stationary emergency flare burns the biogas in the event of a malfunction or if the gas storage tanks can no longer hold any additional biogas. Other applications for the emergency flare include carrying out maintenance work or if the gas cannot be used further due to poor quality. Gas flares are also used when starting up a biogas plant. The gas flare prevents the uncontrolled release of biogas into the environment due to the overpressure protection of the gas storage tanks. Harmful substances are converted into less environmentally hazardous substances, and the methane contained in the biogas is burnt to form water and carbon dioxide. Depending on the type, they can burn biogas with a methane content of 10 to 15% by volume. Stationary plants are usually freestanding but can also be installed on the CHP as a system solution. The materials used are stainless steel or steel, and gas flares are designed for up to 3,000 m³/h. The gas flares are installed directly upstream of the gas flare. A flame arrester must be installed directly in front of the gas flare to prevent open flames from flashing back into the gas-carrying system. They are basically suitable for all biogas plants (Trávníček et al., 2019).

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Figure 34: Emergency flare of a biogas plant [FNR/D. Riesel] (Fachagentur Nachwachsende Rohstoffe, 2013).

1.5 Digestate

1.5.1 Digestate storage

Digestate accumulate in the fermenter. Compared to the substrates used, the digestate has a low dry matter content due to microbial degradation, only a very low oDM content, a higher water content, less structural material and is more homogeneous. The macro- and micronutrient content is almost identical to that of the substrate. Before the digestates can be used on agricultural land in the fertilisation periods (spring and possibly autumn), they must be stored temporarily. The storage capacity to be aimed for depends on local regulations, and in Germany is up to 270 days. Usually, the digestate is separated before storage to reduce the amount to be stored. For practical reasons, digestate storage facilities for solid and liquid digestate are located directly on the premises of a biogas plant. The storage facilities are integrated into the operational processes of a biogas plant via pumps, pipelines, or other conveying equipment. In some cases, they are also built outside biogas plants. In newer plants, a gas-tight cover of the tanks is mandatory to prevent methane emissions. In practice, it sometimes makes sense to combine gas-tight storage tanks for liquid digestate with secondary fermenters. In this case, the digestate storage tanks are only partially emptied and thus used for gas production and digestate storage at the same time.

Solid digestate: These are produced during solid matter fermentation and as a separated (solid) component of the fermentation product of wet fermentation. Solid digestates are stackable and pourable. Depending on the intended use, the digestates are stored in different ways. Storage can take place in the open air or in halls as well as in containers. It is important that the storage facilities meet the requirements of the fermentation product. Per-

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colation liquid and press water must not penetrate the soil. Therefore, the floors of the storage facilities are made of concrete or asphalt. Escaping liquids are collected and returned to the fermenter or the digestate store, for example. Silage camps (see chapter 1.2.2 Delivery and storage of substrates) are used, for example (Weiland, 2010).

An outdoor ground plate is constructed in concrete or mastic asphalt, it is liquid-tight towards the bottom. They are built with a slope for the drainage of liquids. In some cases, they are equipped with backfill walls and a roof. The advantage is that large storage volumes can be realised relatively cheaply. A disadvantage is the loss of nitrogen during long storage periods. If the floor slab is not covered, nutrients are washed out and very high liquid quantities may have to be collected. A storage hall with a concrete floor or mastic asphalt floor is sealed at the bottom. Like the floor slab, it must be able to capture seepage water. The advantage is that it is independent of the weather, the disadvantage is that ventilation is required, and the increased constructional and technical effort compared to outdoor storage. A container, usually made of steel, is another storage option. In contrast to the other storage applications, this one is transportable. If it is not roofed, nutrients may be washed out (Persson et al., 2019).

Liquid digestate: Liquid digestate is stored in earth basins (lagoons) and in cylindrical or rectangular containers. Earth basins are usually rectangular and are embedded in the ground. To prevent emissions, they are covered with a plastic film. Characteristic of this type of storage are the relatively low construction costs for a large volume. More frequently used are containers made of concrete or steel. These can be constructed above or below ground. As described in chapter 1.3.3, fermentation residue stores can be equipped with agitators. These serve to homogenise the digestates before removal (Paolini et al., 2018).

Earth basins are soil pits lined with plastic film, usually rectangular in shape. They are designed with two layers for leakage detection. Agitators can be used here. They are very inexpensive to realise and quick to construct. Construction with large volumes is possible, and maintenance costs are very low. NH_3 emissions can be reduced by using an emission protection foil. A disadvantage is the possible outgassing of CH_4, NH_3 and N_2O, a loss of nitrogen or fertiliser value and, in the case of open earth basins, odour emissions and precipitation can occur. Earth basins can now also be covered with gas storage tanks.

Storage tanks (Figure 35) are usually cylindrical or rectangular, they are available as high or low tanks, and the digestates contained can be homogenised by means of an agitator. They are available in open or closed design, mostly unheated, but also with heating and insulation. The advantage is that they can be covered, so there is no precipitation, which also has a positive effect on any odour emissions. The quality of the fertiliser can be maintained. In a gas-tight design, the containers represent an additional gas reservoir. Open tank systems have the same disadvantages as open earth tanks. Fermentation residue storage usually results in the formation of a floating layer (Figure 34). This is desirable in most cases of open construction, as it reduces odour emissions. Due to the low dry matter content of the liquid digestates, there is no danger of sediments settling on a large scale. There is also no risk of disturbing the gas discharge, as the digestates usually only have a very small gas potential. The stirring technique can therefore be kept much simpler with digestates than with fermenters.

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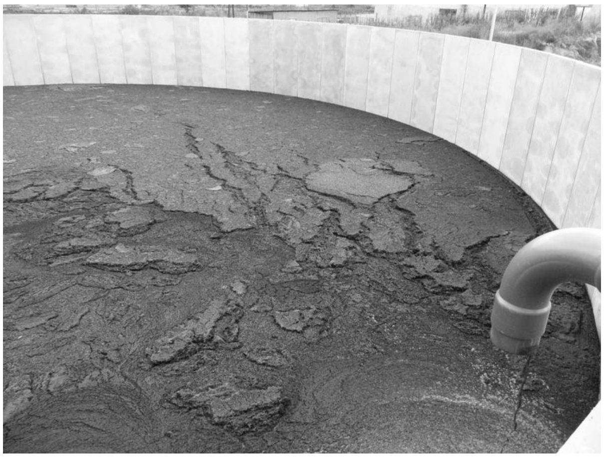

Figure 35: Open construction of digestate storage tank, surface of digestate is forming a dry crust (Vanek, 2011).

1.5.2 Solid/liquid - separation of digestates

Solid's separation: The basic process of digestate treatment is solids separation. Solid's separation makes it possible to reduce the storage volume for liquid fermentation residues and to reduce the formation of sinking and floating layers. In addition, the solids separation enables a separation of the nutrients in the fermentation substrate, the soluble mineral nitrogen remains in the liquid phase, while organically bound nitrogen and phosphorus largely remain in the solid phase. The separated liquid phase can be spread or further processed, the separated solids can be composted or dried.

The properties of the digestate and the configuration of the separator determine the separation efficiency of the processes used. The higher the dry matter content in the digestate, the higher the possible volume reduction (Lukehurst et al., 2011).

Screw presses (Figure 36): Screw presses are used as the first link in the mechanical processes for solids separation or on their own. In the presses, the digestate is pressed against a screen basket by means of a screw (Lindner, 2020). Typical pressure levels of presses are around 30 to 50 bar. In addition to the technical condition of the press, a certain amount of structural material is necessary in the digestate substrate. This forms a plug, which considerably improves the pressing result. Due to the high-pressure levels and the mineral components present in the substrate, screw presses are subject to high levels of abrasion. The screw press is usually installed directly downstream of the fermentation process; the high temperatures and the pH value usually causes the release of ammonia. This can be compensated for by exhaust air collection and treatment.

Good separation efficiencies can be achieved with presses of this type; dry matter contents of up to 40 % are possible.

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Figure 36: Screw presses for dewatering biowaste digestate (Raussen und Kern, 2016).

Decanter / Belt filter presses (Figure 37): Decanters as well as belt filter presses are used for the separation of digestates from wet fermentation plants, as well as for the further dewatering of the liquid digestate after the use of a press screw. When **decanter centrifuges** are used, it is important for a good separation result that the solids have a large density difference to the liquid phase. To improve the dewatering behaviour, flocculants can be added to accelerate the sedimentation or flotation of the suspending particles. Decanter centrifuges achieve a degree of separation of 15 % solid phase and 85 % liquid phase. Decanter cakes have a high density, in this state they are not compostable and must first be made compostable by mixing. **Belt filter presses** are continuous pressure filters with circulating screen cloths as filter media, in which the process sequence takes place in two stages (Lindner, 2020). The first stage is a gravity filtration, the second stage is a squeezing out of the sludge between two screens, which are guided around several rollers. The degree of separation is up to 51 %. A disturbing factor is the large amount of rinsing water required. Both the decanter centrifuges and the belt filter presses are connected downstream of a screw press during digestate preparation. The aim is to process the liquid phase for downstream processes (see chapter 1.5.3 Processing and utilisation of digestates) (Lindner, 2020).

Figure 37: *Decanter (l), belt filter press (r) - for further dewatering of press water from pressed biowaste digestate* (Raussen und Kern, 2016).

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1.5.3 Processing and utilisation of digestates

Often, digestates are not processed further, but used directly as fertiliser. In plants that cultivate energy crops on their own land, it is essential to return the nutrients to their own land. In other cases, for example, when residues from other farms are used for biogas production, there is no internal use for them. Due to the high content of macro- and micronutrients and the large quantity, it is not possible to discharge the liquid phase into the sewage network. In this case, processing the digestate can help to increase economic efficiency. Under certain circumstances, the digestate can be sold as fertiliser; in any case, drying helps to save weight and thus transport costs. This is also a possibility to use waste heat from the CHP. The following drying processes correspond to the state of the art for the treatment of solid digestate. They differ greatly in their prevalence and functional reliability (see **Table 12**). The processes for drying the solid phase are borrowed from other areas of application and have been tested; the degree of adaptation is to be regarded as low.

In other cases, nutrients must be removed from the liquid digestate in order not to overfertilize the soils. In these cases, separation of the nutrients and sale to other regions would be desirable. The processes for treating the liquid phase do not yet correspond to the state of the art, a high need for development is seen here. The membrane process is the most advanced, it is established on the market and is operated in several reference plants. There is still potential in this process, which can reduce energy consumption and wear. Evaporation and stripping processes are not yet far advanced in terms of large-scale continuous operation. In the future, stricter fertiliser regulations will greatly increase the problem locally. **Figure 38** shows an overview of different digestate treatment processes.

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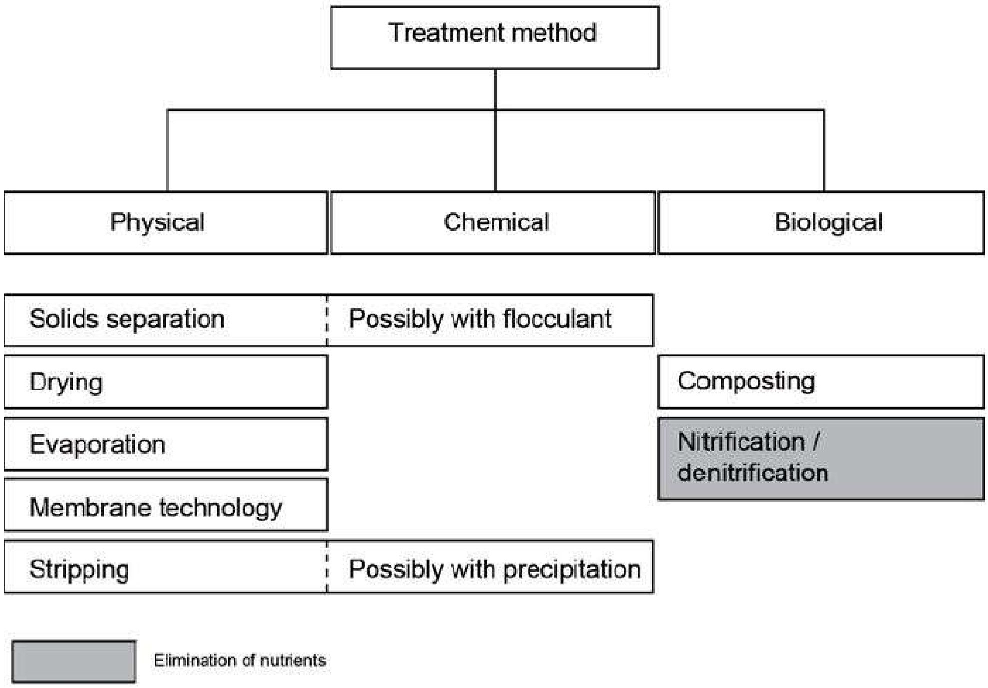

Figure 38: Classification of treatment processes by type (Fachagentur Nachwachsende Rohstoffe, 2010).

Evaporation of liquid digestates: This method is a multi-stage process in which the liquid is first heated and then the temperature is gradually increased to the boiling point under negative pressure. Evaporation under vacuum requires reduced electrical energy compared to evaporation without vacuum. Temperatures of 80 °C are already sufficient in a vacuum (Lindner, 2020). The pH value is lowered by adding acid, this avoids ammonia losses. The amount of fermentation residue is reduced by 70 %. In this process, the fermentation residue is sanitised due to the high temperatures. Up to 4 times higher solids concentrations can be achieved, which significantly reduces storage and transport costs (Lukehurst et al., 2010). Evaporation requires upstream dewatering of the liquid digestate by means of a decanter or belt filter press. Another disadvantage of this process is the high amount of thermal energy required. This process only makes sense if hygienization is necessary, e.g., when using slaughterhouse waste and a lot of excess heat is available.

Drying of solid digestates: For drying, established technology from other areas is used. For this process step, for example, drum, belt, or push-turn dryers can be used. In most processes, heat is transferred by hot air which flows over or through the material to be dried (Lukehurst et al., 2010).

A **belt dryer** (see **Figure 39**) consists of a drying chamber and several conveyor belts. On these, the free-flowing material to be conveyed is usually transported on several conveyor belts running in opposite directions. The belts are permeable to air and are made of wire mesh or perforated steel plates. Hot air flows through the conveyor belts and the material is dried. The material is mixed and homogenised by transferring it to different belts. The

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temperature level of the belt dryers is between 80 - 120 °C, for which dissipated heat from the CHP can be used. The exhaust air must be treated (Lindner, 2020).

A **drum dryer** (see **Figure 39**) consists of a slightly inclined rotary tube with an integrated hot air blower (Lindner, 2020). Mechanically dewatered digestate is fed onto the raised end of the rotary pipe and passes through the rotating pipe on a long spiral track. For this purpose, multiple-pass drum dryers are used. Hot air at more than > 200 °C flows through the drum in co-current or counter-current. For economic reasons, it is advisable to use the waste gas heat of a CHP (Lindner, 2020).

The ammonium contained in the solid phase passes into the drying air as ammonia during drying (Fachagentur Nachwachsende Rohstoffe, 2013). Exhaust air treatment is necessary to prevent ammonia emissions and odour emissions.

Figure 39: Belt dryer for fermentation residues of the biowaste fermentation Leonberg plant (left) Drum dryer for digestate (right) (Raussen and Kern, 2016).

Solid digestate composting: Composting is an aerobic process for treating organic waste. The aim of this process is to stabilise the organic components, kill pathogenic germs and weed seeds and reduce odour emissions. For composting, oxygen must be added to the digestate. The digestate is low-structure material, for successful composting it needs to have high-structure material such as bark mulch added to it and the material needs to be turned over. Only 55 °C is reached during composting, not 75 °C, which is necessary for hygienization. The reduced self-heating is caused by the anaerobic decomposition of carbon within the biogas plant; therefore, the treated material only reaches low temperatures during composting.

Membrane technology for liquid digestate: This process has its origins in wastewater treatment and is used there to treat water with a high organic load. In the field of biogas plants, it can be used to process the liquid digestate to such an extent that it can be discharged into the sewage network. The full treatment process has been adapted for biogas plants and is already in use. A unique feature is that no heat is required for this type of digestate treatment. It can thus be used in plants that do not have any surplus heat (for example, satellite CHPs). In this filtration process, decreasing pore size in combination with reverse osmosis produces a permeate from a liquid that can be discharged into the

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wastewater network and a concentrate enriched with nutrients. Phosphorus is retained in the ultrafiltration and is present in the retentate, while ammonium and potassium accumulate in the concentrate. The permeate is largely free of nutrients and can be discharged as wastewater. In this process, the dry matter content must be very low ≤ 3 %, which requires solid-liquid separation by means of a decanter (Fachagentur Nachwachsende Rohstoffe, 2013). Since the costs for this process are very high on a large scale, it only makes sense if another use of the digestates is ruled out.

Stripping of liquid digestate: Stripping is a process in which substances to be separated are removed from liquids by passing gases through the liquid and the ingredients pass into the gas phase. The aim is usually to reduce the nitrogen content in the liquid digestate to such an extent that it can be used as fertiliser. To support the process, the temperature and the pH value can be increased. In the case of steam stripping, the temperature is increased, and the required gas volume flow decreases with increasing temperature. Desorption then takes place, the ammonia in the gas phase is converted into a disposable or recyclable product. The desorption of ammonia can be done by condensation, washing with acids or by reaction with an aqueous solution of gypsum. Ammonium sulphate is the end-product of desorption. **Table 12** gives an overview of possible processes for digestate treatment.

Table 12: Comparative evaluation of digestate treatment processes (Fachagentur Nachwachsende Rohstoffe, 2013)

	Separation	Drying	Membrane technology	Evaporation	Stripping
Operational reliability	++	+/0	+	0	0
Dissemination status	++	+	+	0	0
Costs	+	+/0	0/-	0	+/0
Product usability					
Solid phase	0	+/0	0	0	0
Liquid (nutrient rich)	0	0	+	+	++
Liquid (nutrient poor)			+	0	0

++ = very good, + = good, o = average, - = poor

1.6 Environmental and plant protection

1.6.1 Avoidance of gaseous emissions and dusts

Gaseous emissions and aerosols: When operating CHP units, the combustion engines release gases such as nitrogen oxides, sulphur oxides and formaldehyde in addition to carbon dioxide. Depending on the type of engine used, its combustion settings and its condition, the quantity and composition of the gases released varies. The quality of the burnt biogas also has an influence on the assessment of engine emissions. The gases are released via the exhaust stack of the CHP unit (Persson et al., 2019).

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Operation-related emission sources can be represented by substrate and digestate storage, open pits or containers, and substrate preparation. Emitters are, for example, methane, ammonia, hydrogen sulphide and nitrous oxide. The amount of emitted gases depends on the construction and process-technological design of the biogas plant. To avoid emissions of gases and dusts, closed systems should be used instead of open systems. Gases may be released if components are leaking, if safety devices are activated or in the event of malfunctions (Persson et al., 2019).

Aerosols are two-substance mixtures of suspended matter and a carrier gas. In biogas plants, there are various sources for the formation of aerosols. Examples include the dumping of dry substrates into storage containers, the improper handling of powdered operating materials, dust swirling up from vehicles or soot particles on the exhaust stack of a CHP unit (ASUE, 2014).

1.6.2 Avoidance of liquid emissions

Liquid emissions: The gases ammonia, carbon dioxide, carbon monoxide, hydrogen sulphide and nitrogen oxides can form aerosols and dissociate in water to form acids or alkalis. This brings the water hazard and possibly corrosive effects to the fore. Other liquid substances can escape due to incorrect storage, leaking components or incorrect handling. These substances can be silage leachate, liquid manure or digestate as well as operating fluids (Liebetrau et al., 2013).

To detect the undesired escape of liquid phases, digesters must be equipped with leakage detection; pipes can be double-walled and equipped with leakage detection. All installations must be carried out professionally and checked for leaks before commissioning and regularly during operation. A wall can be built to collect escaping liquids. The concrete measures required can be derived from local laws and regulations and are determined during the approval process.

1.6.3 Emission reduction measures

For substrate delivery, the substrates should be delivered in closed containers; this applies to odour-intensive and dusty substrates. The roads should be paved, and the biogas plant operator must ensure cleanliness on the premises. When storing the substrate, the silage must be covered; this reduces the seeping juices and dry matter losses. A film can be used for the cover. Dusty and odour-intensive substrates are to be covered and possibly stored in storage halls. Collection containers for the leachate must be installed and, if necessary, fed directly into the fermenter. Slurry systems are to be covered with an odour-mitigating film. The state of the art for substrate introduction is to cover the preliminary pit and the introduction opening. The filling level of all types of gas storage tanks must be monitored. Before the gas storage tank releases gas via the pressure relief valve due to overfilling, the gas utilisation system (CHP or gas emergency flare) must be activated. Safety-relevant components

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such as the supply of supporting air in the case of load-bearing roofs or the compressed air clamping in the case of single-skin foil roofs are designed redundantly and integrated into the emergency power supply. The emergency power supply must be designed in such a way that the supply is guaranteed after 20 minutes at the latest. This can be achieved by automatically starting generators or 24-hour standby service. In the case of load-bearing roofs, the supporting air must be checked regularly for methane content and the entire system should be checked annually for leaks using a methane-sensitive, optical gas camera. For additional gas consumption units, the following applies: before the overpressure safety devices are activated, another gas consumption unit (flare) must automatically go into operation. The maximum biogas flow must be assumed for the entire biogas plant. The over pressure and under pressure safety devices may only be activated after the biogas flare has been operated and in the event of a malfunction. All liquid-carrying pipes in gas-carrying containers must always be submerged to prevent biogas from flowing in. For this purpose, the filling levels must be monitored. This also applies to the condensate line from the gas system.

In the field of biogas utilisation for CHP units, gas boilers, gas turbines, internal engine measures are taken to reduce emissions and exhaust gas purification devices such as oxidation catalysts are installed downstream. For biogas upgrading, post-treatment of the exhaust gas is necessary for all processes (except for amine scrubbing); this can be, for example, thermal post-combustion or catalytic post-combustion. For separation during digestate processing, these should be enclosed and have an exhaust air cleaning system. Ideally, the digestate should be processed immediately, and the solid fraction should be compacted and covered until it is spread. Drying should also be enclosed and have exhaust air purification. If necessary, the digestate storage facilities must be covered gas-tight (strongly dependent on the retention time and the substrate used) and have a connection to the gas utilisation system. A hydraulic retention time of 150 days in the gas-tight system should be observed for substrates that require a long retention time, such as straw. When transporting fermentation residues, the transport should take place in closed containers (Beil et al., 2021).

1.6.4 Noise protection

Noise emissions during the operation of biogas plants are mainly caused by vehicles and the operation of the CHP. One way to minimise noise emissions is to use an automated substrate feed system. This can minimise the frequency of use of wheel loaders or forklifts. The emissions of the CHP unit can be reduced by a soundproof cover and by installing it in closed rooms, halls, or containers. Silencers are installed at the supply and exhaust air openings and at the exhaust pipe. The use of low-noise air coolers as well as the avoidance of structure-borne noise transmission via the exhaust air stack, cooler or motor by means of sound-decoupled mountings are technical measures to reduce noise emissions. Often, sound predictions must be made before approval (Liebetrau et al., 2013) and sound level measurements are carried out on the finished installation to confirm compliance with the limit values.

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Biogas can form an explosive gas mixture in combination with air. Depending on the state variables methane/carbon dioxide content, temperature, pressure, and humidity, they can shift the explosion limits. Above the limits, there is no longer a risk of explosion, but fires can be caused by open fire, switching functions of electrical equipment or lightning. It must therefore be assumed that there is an increased risk of explosion and fire in the vicinity of fermentation tanks and gas storage tanks. Different areas of the plant are therefore legally divided into different "potentially explosive atmospheres". Special labelling, precautionary and safety measures apply to these Ex-zones. The explosion zones of the individual components of biogas are shown in **Table 13**. It is mandatory to draw up a risk assessment documenting where explosive atmospheres may form and describing appropriate countermeasures (Cividino et al., 2014).

Table 13: Properties of biogas components (Fachagentur Nachwachsende Rohstoffe, 2013).

		CH_4	CO_2	H_2S	CO	H
Density	kg/m^3	0,72	1,98	1,54	1,25	0,09
Density relative to air	-	0,55	4,53	1,19	0,97	0,07
Ignition temperature	°C	600	-	270	605	585
Explosion range	Vol.%	4,4-16,5	-	4,3-45,5	10,9-75,6	4-77
Workplace exposure limit (MAC value)	ppm	n. s.	5000	10	30	n. s.

Zone 0

In the hazardous area of zone 0, an explosive atmosphere occurs continuously over a long period of time or predominantly over time (Fachagentur Nachwachsende Rohstoffe, 2013). These zones are generally not found in biogas plants.

Zone 1

In these areas, an explosive atmosphere may occasionally occur during normal operation of a biogas plant. These areas are usually in the immediate vicinity of entry points to the gas storage tank or on the gas-carrying side of the fermentation tanks and in the vicinity of blow-off devices, overpressure protection or gas flares (Fachagentur Nachwachsende Rohstoffe, 2013). In the case of free ventilation, these areas must be provided with safety measures within a radius of 1 m. Only equipment approved for Zone 0 and Zone 1 and explosion-protected equipment may be used here. If Zone 1 occurs in a closed room, Zone 1 extends to the entire room.

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Zone 2

In these zones, an explosive gas-air mixture is not expected to form during normal operation. If such a mixture does occur, it is likely to be very rare and not of long duration (e.g., during maintenance work or in the event of a malfunction). Zone 2 can be found, among other places, at the entry openings and inside the fermenter, and in the case of gas storage tanks, the immediate vicinity of the ventilation openings. In these areas, the measures of zone 2 must be implemented within a radius of 1 to 3 m (Fachagentur Nachwachsende Rohstoffe, 2013).

For zones 0 to 2, no ignition sources may occur within the zones. Sources of ignition are, for example, hot surfaces, naked flames or mechanically or electrically generated sparks. Such areas must be provided with warning and information signs. There are also divisions of explosion protection zones for dusts, but these are of less relevance. They usually play a role if solid, dust-like operating aids are used improperly. This can lead to the formation of explosive atmospheres for a short time. Precautions must be taken to ensure safe storage and handling. For example, packaging and containers with operating materials must be tightly closed and the rooms must be adequately ventilated (Fachagentur Nachwachsende Rohstoffe, 2013).

1.7 Potential for improvement

1.7.1 Technical potential for improvement

There is potential for technical improvement in many areas of biogas production (Theuerl et al., 2019). This includes above all the development of special aggregates adapted to the conditions of a biogas plant. There is a need for development, among other things, of separators for low-structure materials such as liquid manure, for drying plants or for ways to remove nutrients from the digestate in a targeted manner. Above all, there is a lack of specialised methods for digestate processing. Most of the methods used so far have been borrowed from other areas of application and therefore do not yet function optimally. However, due to the tightening of fertiliser guidelines, there will be more demand for targeted digestate treatment in the future.

There is also development potential in the development of novel processes for the fermentation of special input materials such as chicken manure or residues from the food industry. So far, these substrates can usually only be used for biogas production when mixed with other substrates.

1.7.2 Biological improvement potential

There is potential for improvement in biology mainly in the adaptability of microorganisms to certain milieu conditions to increase process stability. Increasing the tolerance to pH-value

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fluctuations and to high ammonium concentrations would be particularly useful here. This would allow the increased use of poultry droppings. This can be obtained cheaply as a residual material from poultry farming, but quickly causes inhibitions in the fermenter due to its high nitrogen content. It would also be economically favourable to improve methane formation at lower temperatures. This would reduce the necessary heating capacity and more heat could be sold.

1.7.3 Potential for organisational improvement

There is potential for organisational improvement mainly in the legal framework for the construction of biogas plants. In recent years, the number of relevant directives and laws has risen sharply. This often leads to confusion even among the specialised authorities and thus hinders approval procedures. In addition, it is becoming increasingly difficult to find companies for individual plant components that can comply with all directives under the given conditions. An example of this is the construction of tanks with an approved system for leakage detection when the local groundwater level is high. Often, compromises must be made in terms of function, which are usually associated with additional costs.

Another point in need of improvement is the feed-in tariff for the electricity produced. Without state subsidies, electricity production in biogas plants is not economically viable. However, by being able to provide electrical energy on demand and with a very short lead time, the biogas sector makes an important contribution to grid stability in regions with a high density of wind and solar plants. In addition, methane emissions can be avoided massively when cattle or pig manure is used. On the other hand, the legal future is often unclear, especially for old plants. At the same time, the national implementation of the EU Directive for Renewable Energies (Directive 2009/28/EC) imposes further fundamental obligations on biogas plant operators.

References

- Aberle J. 2016. Anforderungen an die Ausführung, den Betrieb und die Wartung von Fahrsiloanlagen. *Biogas Forum Bayern Nr. IV - 14/2016.* http://www.biogas-forum-bayern.de/De/Fachinformationen/Technik/16.html (accessed 13.10.2021).
- Alonso-Vicario A, Ochoa-Gómez JR, Gil-Río S, Gómez-Jiménez-Aberasturi O, Ramírez-López CA, Torrecilla-Soria J, Domínguez A. 2010. Purification and upgrading of biogas by pressure swing adsorption on synthetic and natural zeolites. Microporous and Mesoporous Materials. 134(1-3), 100-107.
- Amon T, Bischoff M, Clemens J, Heuwinkel H, Keymer U, Meißauer G. 2015. Gasausbeute in landwirtschaftlichen Biogasanlagen. 3. Aufl. Darmstadt: Kuratorium für Technik und Bauwesen in der Landwirtschaft (KTBL, 107).
- Armatec FTS GmbH & Co. KG. Exzenterschneckenpumpe Biogas und Industrie. https://www.armatec-fts.com/produkte/pumpen/exzenterschneckenpumpe-biogas-und-industrie/ (accessed 20.10.2021).

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- ASUE. 2014. BHKW-Kenndaten 2014/2015. Module, Anbieter, Kosten (Bestellnummer: 05 1014). Essen: Verlag für sparsamen und umweltfreundlichen Energieverbrauch.
- Bachmann ESN. 2013. Design and engineering of biogas plants. In The biogas handbook (pp. 191-211). Woodhead Publishing.
- Bauer C, Lebuhn M, Gronauer Andreas. 2009. Mikrobiologische Prozesse in landwirtschaftlichen Biogasanlagen. Freising-Weihenstephan: Bayerische Landesanstalt für Landwirtschaft (LfL). ISSN 1611-4159.
- Beil M, Beyrich W, Holzhammer U, Krause T. 2021. Biomethan. Gülzow : Fachagentur Nachwachsende Rohstoffe e. V.: https://www.fnr.de/fileadmin/Projekte/2021/Mediathek/fnr_biomethan_web.pdf (accessed 10.06.2022).
- Bekon GmbH. The Bekon Process. https://www.bekon.eu/en/technology/ (accessed 18.10.2021).
- BioBG. https://www.biobg.de/produkte/aktivkohlefilter/ (accessed 25.10 2021).
- Biogas-Netzeinspeisung: https://www.biogas-netzeinspeisung.at/technische-planung/biogasgestehung/gasertraege2.html (accessed 07.10.2021).
- Biomin. Entschwefelung in Biogasanlagen. https://www.biomin.net/de/science-hub/entschwefelung-in-biogasanlagen/ (accessed 10.12.2020).
- Boe K, Batstone, Damien J, Steyer J, Angelidaki I. 2010. State indicators for monitoring the anaerobic digestion process. In: Water research 44 (20), S. 5973–5980.
- Brehmer M, Kraume M. 2016. Experimental study to develop a control system for submersible mixers in biogas plants. Czasopismo Techniczne, 2016 (Mechanika Zeszyt 1-M (1) 2016), 33-42.
- Bundesforschungsanstalt für Landwirtschaft. 2005. Ergebnisse des Biogas-Messprogramms. Gülzow: Fachagentur Nachwachsende Rohstoffe e. V. (FNR).
- Chandra A, Tiwari GN, Srivastava VK, Yadav YP. 1991. Performance evaluation of biogas burners. Energy conversion and management, 32(4), 353-358.
- Chae KJ, Jang AM, Yim SK, Kim IS. 2008. The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. Bioresource technology, 99, 1-6.
- Chen XY, Vinh-Thang H, Ramirez AA, Rodrigue D, Kaliaguine S, 2015. Membrane gas separation technologies for biogas upgrading. Rsc Advances. 5, 24399-24448.
- Cividino SRS, Malev O, Lacovig M, Pergher G, Dell'Antonia D, Gubiani R, Vello M. 2014. BiogasAgriAtex, new methods of risk assessment explosion on biogas plants. Appl Math Sci, 8, 6599-6619.
- Clarke WP. 2018. The uptake of anaerobic digestion for the organic fraction of municipal solid waste–push versus pull factors. Bioresource technology, 249, 1040-1043.
- Deepanraj B, Sivasubramanian V, Jayaraj S. 2014. Biogas generation through anaerobic digestion process-an overview. Research Journal of Chemistry and Environment, 18, 5.
- DBFZ Deutsches Biomasseforschungszentrum. 2021. BIOGAS-MESSPROGRAMM III, Fachagentur Nachwachsende Rohstoffe e. V. (FNR)
- Dobre P, Nicolae F, Matei F. 2014. Main factors affecting biogas production-an overview. Romanian Biotechnological Letters. 19, 9283-9296.
- Dutton, John A. https://www.e-education.psu.edu/egee439/node/727. https://www.e-education.psu.edu/egee439/node/727 (accessed 08.10.2021).
- Energypedia. https://energypedia.info/wiki/Biogas_Recovery: https://energypedia.info/wiki/Biogas_Recovery (accessed 15.10.2021).
- Effenberger M, Kissel R, Lehner A, Gronauer A. 2008. Verfahrenstechnische Bewertung landwirtschaftlicher Biogasanlagen – Auslastung und energetische Effizienz/Technical Assessment of Agricultural Biogas plants – Utilization Ratio and Energy Efficiency. Landtech/Agric Eng 63, 290-292a.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Effenberger M, Lebuhn M. 2011. Repowering von Biogasanlagen zur Steigerung der Prozesseffizienz. KTBL-Schrift, Biogas in der Landwirtschaft: Stand und Perspektiven. Darmstadt, Germany: Kuratorium für Technik und Bauwesen in der Landwirtschaft.
- Fachagentur Nachwachsende Rohstoffe e.V. (FNR). 2010. Guide to Biogas - From production to use. Gülzow.
- Fachagentur Nachwachsende Rohstoffe e. V. (FNR) (Hg.) (2009): Biogasmessprogramm II. 61 Biogasanlagen im Vergleich. Johann Heinrich von Thünen-Institut; Institut für Agrartechnologie und Biosystemtechnik; Fachagentur Nachwachsende Rohstoffe. 1. Aufl. Hannover, Gülzow bei Güstrow: Technische Informationsbibliothek u. Universitätsbibliothek; Fachagentur Nachwachsende Rohstoffe: https://edocs.tib.eu/files/e01fb10/62358767X.pdf (accessed 12.06.2022)
- Fachagentur Nachwachsende Rohstoffe e. V. (FNR). 2013. Leitfaden Biogas - Von der Gewinnung zur Nutzung. Gülzow.
- Fachagentur Nachwachsende Rohstoffe e. V. (FNR). 2018. Flexibilisierung von Biogasanlagen. Gülzow.
- Fu Y, Luo T, Mei Z, Li J, Qiu K, Ge Y. 2018. Dry anaerobic digestion technologies for agricultural straw and acceptability in China. Sustainability. 10, 4588.
- Fuchs W, Wang X, Gabauer W, Ortner M, Li Z. 2018. Tackling ammonia inhibition for efficient biogas production from chicken manure: Status and technical trends in Europe and China. Renewable and Sustainable Energy Reviews. 97, 186-199.
- Gomez, Claudius da Costa (Fachverband Biogas e.V.). 2016. Biogas Wissen Kompakt. Freising : Fachverband Biogas: https://issuu.com/fachverband.biogas/docs/biogas_wissen_kompakt_versand (accessed 09.06.2022)
- Hopfner-Sixt K, Amon T, Walla C, Pötsch EM, Amon B, Milovanovic D, Weichselbaum W. 2007. Analyse und Optimierung neuer Biogasanlagen. Österreichische Forschungsförderungsgesellschaft (FFG).
- Khan IU, Othman MHD, Hashim H, Matsuura T, Ismail AF, Rezaei-DashtArzhandi M, Azelee IW. 2017. Biogas as a renewable energy fuel–A review of biogas upgrading, utilisation and storage. Energy Conversion and Management, 150, 277-294.
- Kissel R, Kliche R, Streicher G, Effenberger M. Empfehlungen für die Auswahl von Rührwerken für Gärbehälter und Gärrestlager. In. Biogas Forum Bayern Nr. IV – 10/2014 (2. Auflage). ALB Bayern e.V. http://www.biogas-forum-bayern.de/publikationen/Empfehlungen_fur_die_Auswahl_von_Ruhrwerken_fur_Garbehalter_und_Garrestlager_2_Auflage.pdf. (accessed 19.10.2021).
- Koch K, Post M, Auer M, Lebuhn M. 2017. Einsatzstoffspezifische Besonderheiten in Der Prozessführung. In Biogas Forum Bayern.
- Krieg A, Fischer T. 2001. Biogas-Anlagentechnik–speziell Vergärung von NAWAROS. In Proceedings of the Conference on Biogas-Fachtagung, Geichlingen-Eifel September (Vol. 1).
- Liebetrau J, Reinelt T, Clemens J, Hafermann C, Friehe J, Weiland P. 2013. Analysis of greenhouse gas emissions from 10 biogas plants within the agricultural sector. Water Sci Technol. 67, 1370-1379.
- Lindner JP. 2020. Rührend für die Umwelt, Prozesstechnische Auslegung von Rührwerken für Biogasfermenter - Teil 1: Rührwerksaufgaben und Rheologie. https://doi.org/10.1002/citp.202001027.
- Lukehurst CT, Frost P, Al Seadi T. 2010. Utilisation of digestate from biogas plants as biofertilizer. IEA bioenergy. 1-36.
- Mohammadrezaei R, Zareei S, Behroozi-Khazaei N. 2018. Optimum mixing rate in biogas reactors: Energy balance calculations and computational fluid dynamics simulation. Energy. 159, 54-60.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Mutungwazi A, Mukumba P, Makaka G. 2018. Biogas digester types installed in South Africa: A review. Renewable and Sustainable Energy Reviews, 81, 172-180.
- Nwokolo N, Mukumba P, Obileke K, Enebe, M. 2020. Waste to Energy: A Focus on the Impact of Substrate Type in Biogas Production. Processes. 8, 1224. https://doi.org/10.3390/pr8101224.
- Ogunlude P, Abunumah O, & Orakwe I, Shehu H, Muhammad-Sukki F, Gobina E. 2019. Comparative evaluation of the effect of pore size and temperature on gas transport in nano-structured ceramic membranes for biogas upgrading. WEENTECH Proceedings in Energy. 195-205. 10.32438/WPE.8319.
- Okoro OV, Sun Z. 2019. Desulphurisation of biogas: a systematic qualitative and economic-based quantitative review of alternative strategies. ChemEngineering. 3, 76.
- Paolini V, Petracchini F, Segreto M, Tomassetti L, Naja N, Cecinato A. 2018. Environmental impact of biogas: A short review of current knowledge. Journal of Environmental Science and Health, Part A, 53(10), 899-906.
- Persson Emelie, Westerholm M, Schnürer A, Nordin A, Tamm D, Nordberg U. 2019. Dry anaerobic digestion of food waste at mesophilic and thermophilic temperature. Stockholm. ISBN 978-91-7673-609-8.
- Planet Biogas: https://planet-biogas.com/en/function-of-a-biogas-plant/ (accessed 07.10.2021).
- Postel J, Jung U, Fischer E, Scholwin F. 2009. Stand der Technik beim Bau und Betrieb von Biogasanlagen. Umweltbundesamt. ISSN 1862-4804.
- Ramos I, Pérez R, Fdz-Polanco M. 2013. Microaerobic desulphurisation unit: A new biological system for the removal of H2S from biogas. Bioresource technology, 142, 633-640.
- Raussen T, Kern M. 2016. Optimale Aufbereitung von Gärresten aus der Bioabfallbehandlung – Ein Leitfaden. Stuttgart : Ministerium für Umwelt, Klima und Energiewirtschaft Baden-Württemberg.
- Rico C, Rico JL, Tejero I, Muñoz N, Gómez B. 2011. Anaerobic digestion of the liquid fraction of dairy manure in pilot plant for biogas production: Residual methane yield of digestate. Waste management. 31, 2167-2173.
- Roskatski K. 2019. Rührwerke in Biogasanlagen–Technik mit zentraler Bedeutung., Gülzower Fachgespräche, 26.
- Schnürer A. 2016. Biogas Production. Microbiology and Technology. In: Advances in biochemical engineering/biotechnology 156, S. 195–234. DOI: 10.1007/10_2016_5.
- Streicher G, Kliche R, Buschmann A, Pohl A, Effenberger M. 2016. Monitoring von Biogasanlagen - Diversifizierung der Einsatzstoffe und Verfahrenstechnik. Abschlussbericht an das Bayerische Staatsministerium für Ernährung, Landwirtschaft und Forsten (FKZ: EW/12/03). Bayerische Landesanstalt für Landwirtschaft (LfL), Freising.
- Strobl M, Keymer U, .2016. Biogasausbeute mobil. Hg. v. Bayerische Landesanstalt für Landwirtschaft. München: https://www.lfl.bayern.de/appl/biogas/ausbeute/ (accessed: 13.02.2020).
- Tan Y, Nookuea W, Li H, Thorin E, Yan J. 2017. Cryogenic technology for biogas upgrading combined with carbon capture-a review of systems and property impacts. Energy Procedia. 142, 3741-3746.
- Theuerl S, Herrmann C, Heiermann M, Grundmann P, Landwehr N, Kreidenweis U, Prochnow A. 2019. The Future Agricultural Biogas Plant in Germany. A Vision. Energies. 12, 396. doi: 10.3390/en12030396.
- Theuerl S, Klang J, Prochnow A. 2019. Process disturbances in agricultural biogas production—Causes, mechanisms and effects on the biogas microbiome: A review. Energies. 12, 365.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Trávníček P, Kotek L, Nejtek V, Koutný T, Junga P, Vítěz T. 2019. Flare stacks on agricultural biogas plants–safety and operational requirements. Research in Agricultural Engineering. 65, 98-104.
- Vanek M. 2011. Odor control in biogas plant – case study. 10.5593/SGEM2015/B41/S17.046.
- Veluchamy C, Gilroyed BH, Kalamdhad AS. 2019. Process performance and biogas production optimizing of mesophilic plug flow anaerobic digestion of corn silage. Fuel. 253, 1097-1103.
- Verein Deutscher Ingenieure (2006): Vergärung organischer Stoffe. Substratcharakterisierung, Probenahme, Stoffdatenerhebung, Gärversuche. Berlin: Beuth (VDI-Richtlinien, 4630).
- Verein Deutscher Ingenieure (2008): Gütekriterien für Biogasanlagen. Dezember 2008. Berlin: Beuth (VDI-Richtlinien, 4631).
- Vo TT, Wall DM, Ring D, Rajendran K, Murphy JD. 2018. Techno-economic analysis of biogas upgrading via amine scrubber, carbon capture and ex-situ methanation. Applied energy, 212, 1191-1202.
- Weiland P. 2010. Biogas production. Current state and perspectives. In: Applied microbiology and biotechnology 85 (4), S. 849–860. doi: 10.1007/s00253-009-2246-7.
- Weinrich, S., Paterson, M., & Roth, U. (2020). Leitfaden zur Substrat-und Effizienz-bewertung an Biogasanlagen. DBFZ Report, (35), 68.
- Wilken D. et al. *Biogas aus Bioabfall.* Freising : Fachverband Biogas e. V. (FvB), 2019.
- Xiao Y, Yuan H, Pang Y, Chen S, Zhu B, Zou D, et al., 2014. CO_2 removal from biogas by water washing system. Chinese Journal of Chemical Engineering. 22, 950-953.

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2. Technological Description

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Abstract: **Waste disposal and green waste:** In Germany, there are almost 700 plants to produce biogas from waste, and many more throughout Europe. Depending on the composition, the substrate properties of waste vary greatly. Technical challenges often exist in the processing of the waste. Special legal requirements also must be taken into account. Advantages, on the other hand, include low-cost purchasing and, in some cases, very high gas yields.

Agriculture and Livestock: Due to the widespread use of biogas plants that are operated with animal residues and energy crops, many findings on the technical requirements have been documented. In plants for the utilization of energy crops, care must be taken to ensure that the DM content and space load are not selected too high; the retention time should be about 60 days. If mainly animal residues are used, too high DM contents and overloading are usually not to be feared. The retention time can be chosen smaller than in other plants, often about 40 days are sufficient.

2.1 The goals of fermentation

The goal of fermentation is usually the production of biogas for the generation and sale of electricity and heat. Even in plants where the biogas is upgraded to biomethane for injection into the natural gas grid, the production of biogas is the primary goal. Accordingly, the substrates are selected and used in such a way that the desired amount of biogas can be produced at the lowest possible cost for the substrates. Downtime due to process disturbances and fluctuating gas production is to be avoided, and stable gas production is to be aimed for. The produced gas quality is also important, for use in combustion engines CHP the biogas must have a methane content of at least 45%. Also, the hydrogen sulphide content should not be too high, otherwise the biogas plant will be damaged. An important side effect in plants for the utilization of cattle or pig manure is the avoidance of methane emissions that would result from storage and direct spreading.

However, in the case of plants for the anaerobic treatment of residual materials and waste, the focus is sometimes actually on waste disposal. In these cases, the production of biogas is only a welcome side effect that makes the process more economical.

2.2 Technical parameters of operation management

The following parameters can be used to obtain detailed information about the processes taking place in a biogas plant. These must be recorded, collected, and evaluated on a plant-

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specific basis. They serve to avoid process disturbances, to recognize trends, to increase plant safety and to optimize plant operation. A distinction can be made between control parameters for early detection of process disturbances (for example, volatile organic acids, FOS/TAC ratio, redox potential, hydrogen in the gas and liquid phases) and control parameters for assessing the process state (for example, substrate composition and quantity, gas production and gas composition, toxicity, fermentation temperature, DM content and viscosity of the fermentation mixture, and pH value) (Drosg, 2013).

2.2.1 Space load

The space load (**Figure 40**) indicates the amount of organic DM (oDM) in kilograms that can be fed to the digester per working volume per unit of time (Fachagentur Nachwachsende Rohstoffe, 2013).

Formula 1: Organic loading rate B_R

$$B_R = \frac{\dot{\mathrm{m}} * \mathrm{c}}{V_R * 100} \; [kg\ oDM\ m^{-3}\ d^{-1}]$$

$\dot{m} = Quantity\ of\ substrate\ supplied\ per\ unit\ of\ time\ \left[\frac{kg}{d}\right];$

$c = Organic\ matter\ concentration\ [\%\ oDM];$

$V_R = Reactor\ volume\ [m^3]$

The mass of the fed substrates is normally recorded by the feeder via built-in weighing elements in the case of solid substrates, or via flow meters upstream of the pumps in the case of liquid substrates. The DM content in the substrate must be continuously determined by random sampling, this also serves quality assurance purposes. Mobile measuring devices are usually available for this purpose at biogas plants. There are different balance limits for the room load. One level is the gas-tight, insulated fermentation tank, another is the total system (sum of the working volumes of all levels) and with or without inclusion of material recirculation. Depending on the reference variables, different space loads result. To be able to compare different biogas plants, it is recommended to specify the space load for the total system without material recirculation (Drosg, 2013). Which space load is chosen for the plant depends on the corresponding conditions. In general, an operator will choose the space load as high as possible to operate his plant effectively. Limits are set by the existing plant technology (performance of pumps, agitators and other aggregates), the maximum permitted biogas production (usually determined with the permit, together with the maximum amount of substances used), the time the substrates need in the fermenter to be degraded (retention time) and the load capacity of the microorganisms. As a result of the material transport through the fermenter, these are discharged with the fermentation residue. If more microorganisms are discharged than can form in the same period of time, their number decreases

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and with it the biological activity in the fermenter. Since the methane-forming archaea in the microbiological community of a fermenter are usually the organisms that grow the slowest, methane formation then declines. Due to the lack of degradation of the acidic degradation products of the preceding biological processes, the pH then begins to decrease, further inhibiting the activity of the organisms. This is referred to as tilting of the fermenter. If the substrate supply (and thus the discharge of microorganisms from the fermenter) is not stopped immediately, the process is irreversible, and the fermenter must be restarted over a long period of time. How far the room load can be increased without tipping is a matter of experience. Here, operators can approach an optimal value in small steps over long periods of time (several months). The room load can also be specified in dimensions other than $kg\ oDM\ m^{-3}\ d^{-1}$. For example, the mass of volatile fatty acids (VS) can be used instead of the DM content $kg\ VS\ m^{-3}\ d^{-1}$. The adjustment of the space load is adjusted at the end via the added substrate quantity per time (Schlegel et al., 2008).

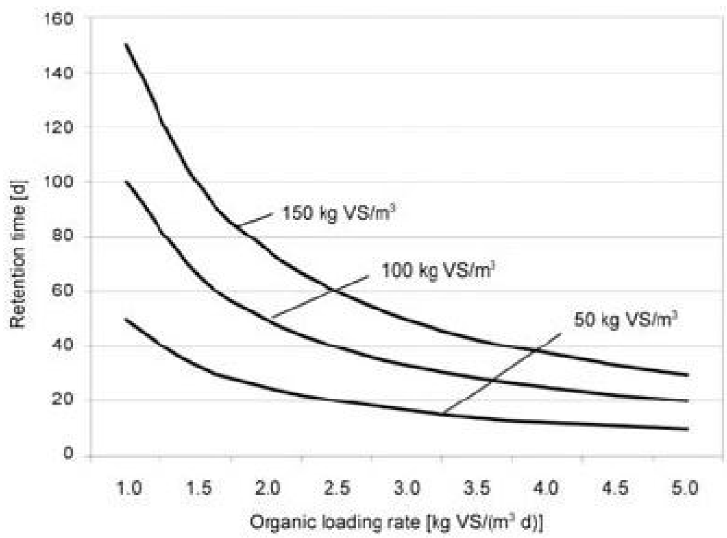

Figure 40: Correlation between organic loading rate and hydraulic retention time for various substrate concentrations (Fachagentur Nachwachsende Rohstoffe, 2010a).

2.2.2 Retention time

The hydraulic retention time is the period that a substrate remains in the digester on a calculated average and is directly dependent on the space load.

Formula 2: Hydraulic retention time

$$\boldsymbol{\tau = \frac{V_R}{\dot{V}}\ [h]}$$

$\tau = HRT = \ Hydraulic\ retention\ time;$

$V_R = Reactor\ volume\ [m^3];\ \dot{V} = Volume\ of\ substrate\ fed\ per\ unit\ time\ [\frac{m^3}{h}];$

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The composition of the substrates has a high impact on the required hydraulic retention time. Likewise, the temperature has a significant influence on the HRT. The dependence between temperature and residence time can be seen in Figure 2. At higher temperatures the residence time decreases, at lower temperatures it increases. In the initial phase, the relative gas yield is sharply increasing and flattens with increasing residence time. The residence time is selected so that the gas yield is as high as possible. The relationship between space loading and residence time is shown in **Figure 41**. For a constant substrate composition, as the space load increases, more input is added to the fermenter and the residence time decreases. The reactor contents are constantly exchanged, and the residence time should be selected so that no more microorganisms leave the fermenter via the effluent mass than can regrow in it. If the residence time is too short, the microorganisms cannot decompose the introduced substrate, which reduces the gas yield. For this reason, the residence time must be adapted to the substrate used. If the amount of substrate added and its composition are known, the reactor volume can be calculated for a given residence time (Fachagentur Nachwachsende Rohstoffe, 2013).

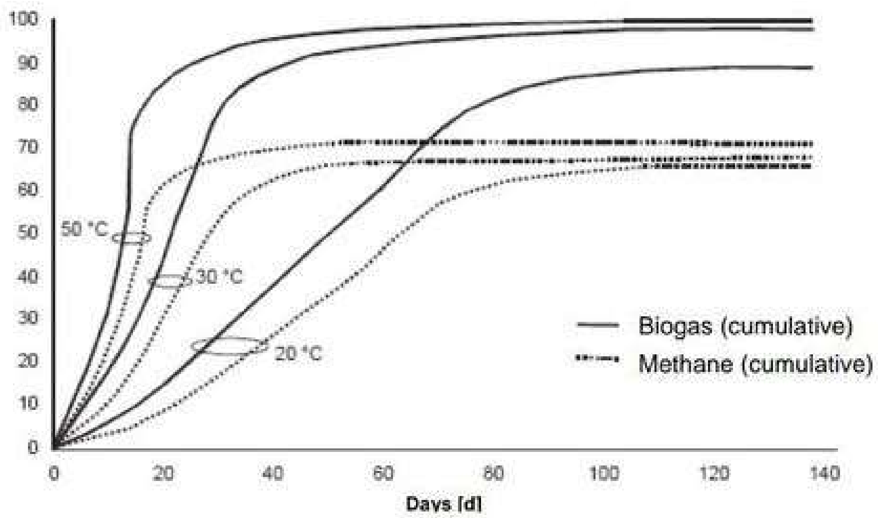

Figure 41: Relative biogas yields, depending on temperature and retention time (Out, 2020).

2.2.3 Productivity, yield and degree of degradation

If the gas production is related to the fermenter volume, this corresponds to the methane productivity. It is defined as the quotient of the daily gas production and the reactor volume, and thus provides information about the effectiveness (Schlegel et al., 2008). The productivity can refer to the biogas (P_{biogas}) as well as to the methane (P_{CH4}).

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Formula 3: Methane productivity

$$\boldsymbol{P_{CH_4} = \frac{\dot{V}_{CH_4}}{V_R}[Nm^3 * m^3 * d^{-1}]}$$

$$\dot{V}_{CH_4} = Methane\ production\ \left[\frac{Nm^3}{d}\right]; V_R = Reactor\ volume\ [m^3]$$

If the gas production is related to the input substances, this results in the yield. It is defined as the quotient of the amount of gas produced, and the organic matter added (Fachagentur Nachwachsende Rohstoffe, 2013). It provides information on the efficiency of methane production from the substrates fed into the digester. As an individual parameter, the yield is not very meaningful since the effective load of the fermenter has no influence in this respect. For a better understanding of the process, the yield should therefore always be stated together with the space load.

Formula 4: Methane yield

$$\boldsymbol{A_{CH_4} = \frac{\dot{V}_{CH_4}}{\dot{m}_{oDM}}[Nm^3 * t^{-1} * oDM]}$$

$$\dot{V}_{CH_4} = Methane\ production\ [Nm^3 * d];\ \dot{m}_{oTS} = Added\ organic\ dry\ matter\ [\frac{t}{d}]$$

The degree of degradation η_{oDM} describes the efficiency of utilization of the substrates used.

Formula 5: Biomass degradation rate

$$\boldsymbol{\eta_{oTS}} = \frac{\boldsymbol{oDM_{sub} * m_{in} - oDM_{Abl} * m_{out}}}{\boldsymbol{oDM_{out} * m_{in}}} * \boldsymbol{100}[\%]$$

$$oDM_{sub} = organic\ dry\ matter\ content\ of\ the\ substrate\ \left[\frac{kg}{t}\right];$$

$$m_{in} = Mass\ of\ fresh\ mass\ added\ [t];$$

$$oDM_{out} = organic\ dry\ matter\ content\ of\ the\ fermenter\ effluent\ [\frac{kg}{t}];$$

$$m_{out} = Mass\ of\ the\ fermentation\ residue\ [t];$$

2.2.4 Gas composition, gas volume measurement and gas analysis

The gas composition of biogas can be taken from **Table 14**. Like the gas production, the gas composition depends very much on the load of the digester and the composition of the substrates used. If the gas production or methane content decreases with a constant feed, this indicates a disturbance or inhibition of the processes. Biogas quantity measurement and

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biogas composition measurement are mandatory on biogas plants and are best suited for process control, as they are measured continuously and react relatively quickly.

Table 14: Composition of biogas (Fachagentur Nachwachsende Rohstoffe, 2013).

Gas	Concentration
Methane (CH_4)	50–75 Vol.-%
Carbon dioxide (CO_2)	25–45 Vol.-%
Water (H_2O)	2–7 Vol.-% (20–40 °C)
Hydrogen sulphide (H_2S)	20–20,000 ppm
Nitrogen (N_2)	< 2 Vol.-%
Oxygen (O_2)	< 2 Vol.-%
Hydrogen (H_2)	< 1 Vol.-%

Gas production at biogas plants is not always measured directly by a flow meter. It can also be determined indirectly by the filling level of the gas storage tanks and the output and running time of the CHP units. Since their gas consumption is known, this is sufficient to infer the amount of gas produced. The filling level of the gas storage tanks is important to be able to control the operating times of the CHP units. In double diaphragm roofs, the level is often determined by means of a tensioning cable with a rod and angle sensor (Lossi and Pütz, 2021). Another method is to use a tension cable with pulley, measuring weight and proximity sensors (Lossi and Pütz, 2021). These mechanical systems have proven themselves in practice and offer the advantage of eliminating ignition sources within the roof. Other systems for detecting the height of the gas storage membrane are based on ultrasound or distance measurement with lasers. In practice, these systems have proven to be more vulnerable and less reliable.

In some cases, the gas volume flow is measured before entering the gas control section in the CHP unit. To obtain good measurement results, the gas should already be dried. Thermal probes are used for the measurement. Mechanical volumetric flow meters are less suitable due to the moisture in the gas. The gas analysis is carried out either with mobile gas measuring instruments for individual monitoring of the containers, or it is carried out in a central recording before entering the gas control line. The stationary gas measuring instruments usually record the parameters CH_4, CO_2, O_2, H_2S.

The methane concentration is the most important component of biogas for the evaluation of the operation management. It represents the combustible fraction of biogas and directly influences the calorific value. The higher the proportions of carbon dioxide and nitrogen, the more the calorific value of the gas is reduced.

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The gas composition and thus the methane content is mainly dependent on the substrate used; the composition can only be influenced to a limited extent by controlling the process. The methane concentration depends on the process parameters such as the fermentation temperature, the load condition of the reactor and the hydraulic retention time as well as by process disturbances and procedures such as biological desulfurization (Weiland, 2010).

2.2.5 Fermentation temperature

Fermentation is an exothermic process, nevertheless, to maintain a defined temperature level, the fermentation tanks must be equipped with heating. The temperature must be measured during fermentation of the substrates. A rapid change of the temperature results in a continuous disturbance of the process biology, due to which the temperature is not suitable as a variable for process control. The temperature inside the fermenter has a strong effect on the environmental conditions. With an increasing temperature, the balance between ammonia and ammonium shifts towards ammonia. This has an inhibitory effect on the acetoclastic methane bacteria and thus has negative consequences, especially when processing protein-rich substrates. Therefore, the temperature of the anaerobic degradation processes must be monitored. Temperatures below 40 °C slow down the biology within the fermenters, the retention time increases, at above 50 °C fewer bacterial strains are present, and the overall process becomes more prone to failure (Song et al., 2004). Temperature sensors are placed at different heights of the fermenter.

2.2.6 Dry matter content and viscosity of the fermentation mixture

The DM content and viscosity are important parameters with regard to the mixing, pumpability and gas discharge of the digested sludge. If the gas bubbles in the digester cannot escape, this results in foam formation. The DM value does not correlate directly with viscosity, yet it is monitored in many plants (Krieg and Fischer, 2001). Direct measurement of the viscosity of the fermentation substrates is very difficult, but it can be measured indirectly via the power input of the agitators. This is possible because the power input of the agitators depends, among other things, on the viscosity of the mixture to be agitated. Normally, the agitators are controlled via a fixed speed or via a predefined time interval. Automatic control of the agitators is state of the art. Nevertheless, manual intervention is also necessary, for example if the viscosity is too high, it must be mixed with fermentation substrate with a very low DM content or with process water from the biogas plant or fresh water. The fermenter is fitted with sight glasses, which are used for visual inspection of the fermenter contents. Associated with this is the problem of foam formation. This is the result of reduced surface tension, which is caused by surface-active substances (Koch et al., 2017). The exact causes of foam formation in a biogas plant are often not known. It occurs more frequently, for example, when spoiled silage is used. It is possible that surface-active intermediates or bacterial groups in interaction with increased gas production are the cause. Foam can clog the gas

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lines and increase the pressure in the fermenter, which can trigger the overpressure protection or even destroy the roof (Fachagentur Nachwachsende Rohstoffe, 2013). The state of the art in digesters is the monitoring of the filling level and, to some extent, the formation of foam. Pressure sensors, conductivity sensors and occasionally infrared sensors are used for this purpose. It is controversial whether measurement of foam formation is useful; these can create a false sense of security. In the event of a biological overreaction occurring and the foam sensor responding, only a few minutes remain before foam enters the gas sampling system. In extreme cases, even the foil roof can tear open. If there is no foam sensor, charging processes that lead to an overdrive of the biogas plant must be avoided (Lossi and Pütz, 2021).

2.2.7 FOS/TAC

The FOS/TAC determination is a titration test for determining the quotients of acid concentration and buffer capacity in the fermentation residue (Lili et al., 2011). The FOS/TAC value therefore provides knowledge about the degradation performance of the fermenter. FOS stands for Volatile Organic Acids, unit [mg/l acetic acid equivalents] and TAC for Total Inorganic Carbonate (alkaline buffer capacity, unit [mg $CaCO_3$/l). The value is a measure of the acidification risk of a plant. With the help of the FOS/TAC, process disturbances can be detected at an early stage and a reaction can be taken if necessary (Reinhold, accessed 04.11.2021). The determination is carried out in external laboratories and not in the biogas plant. However, a contract with an appropriate laboratory for sampling and control is not unusual.

As the percentage of organic acids in a digester increases, the methane-producing bacteria are inhibited. With decreasing pH, the inhibition effect increases (at pH >7.4). However, the pH value is only suitable for accurate process analysis to a limited extent, since the acids in the fermenter are initially buffered. In contrast to the pH value, the FOS/TAC value detects the presence of the buffer (Postel et al., 2009).

The determination can be done by manual titration or by titrator. A measurement is carried out in the first step by drawing a representative fermentation substrate sample. In a further step, the sample is freed from coarse components (e.g., by means of a filter or a centrifuge). A defined amount of substrate is taken and immersed in an electrode. The sample is placed on a magnetic stirrer and permanently homogenized. Now titration with 0.1 N H_2SO_4 follows until a pH value of 5 is reached. The consumed acid is recorded. This step is repeated until a pH value of 4.4 is reached. The value can then be calculated according to formula 8 (Reinhold, accessed 04.11.2021).

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Formula 6: Determination FOS

$$FOS = \frac{20 * ((FP_2 - FP_1 * 1{,}66 - 0{,}15) * f_{H_2SO_4} * 500}{sample\ size}$$

Formula 7: Determination TAC

$$TAC = \frac{20 * FP_1 * f_{H_2SO_4} * 250}{sample\ size}$$

Formula 8: Determination FAS/TAC Ratio

$$FOS/TAC = \frac{FOS}{TAC}$$

$$FOS:\ free\ organic\ acids; FP_1 = fixed\ end\ point\ up\ to\ pH\ 5.0;\ FP_2 = fixed\ end\ point\ up\ to\ pH\ 4.4; 1.66 = \ free\ organic\ acids; 0.15 = \ free\ organic\ acids; 500 = \ free\ organic\ acids;\ \ f_{H_2SO_4} = Titer\ from\ c(H_2SO_4) = 0.05\frac{mol}{L}; 250 = \ free\ organic\ acids$$

A FOS/TAC ratio of 0.3 to 0.4 is considered normal in practice, but this value is fluctuating in practice because it is plant-specific; there is a strong dependence on the substrate composition. In Energy crops plants, the value is between 0.4 and 0.6 with stable process control (Singlitico et al., 2017).

The smaller the amount of acid required until the pH value of 5.0 is reached, the lower the buffer capacity of the substrate. If there are few organic acids in the fermenter, the more acid addition is needed to reach a pH of 5.0. The higher the basic buffer, the more acid is needed to get to a pH of 5.0. A high starting pH above 7.9 is indicative of increased NH4-N. A high FOS value means that there are more organic acids in the digester, the higher the FOS value, the more acid is needed to get to a pH starting at 5.0 to a target pH of 4.4. Not only is the FOS/TAC ratio required for accurate interpretation, but the individual trends for FOS and TOC must also be considered for accurate analysis. For example, if the FOS/TAC ratio is thought to be less than 0.5, it may be that the organic acid content is too high, and they are merely well buffered. **Table 15** gives an overview of the FOS/TAC ratios, their significance, and the resulting measures.

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Table 15: The relationship between the FOS/TAC value and the condition of the plant, as well as the measures required to balance the acing of the plant (Lossie and Pütz, 2021).

FOS/TAC Value	Meaning	Measures
>0,6	Plant heavily overfed	Stop feeding
0.5-0.6	Plant overfed	Restrict feeding
0.4-0.5	Plant heavily loaded	Increase observation
0.3-0.4	Plant utilized to capacity	Keep feeding as is
0.2-0.3	Plant hungry	Slowly increase feeding
<0.2	Plant very hungry	Increase feeding rapidly

2.2.8 pH value and volatile fatty acids

The optimum pH environment for the microorganisms across all process stages is between 6.4 - 8. Within this operating window, a good gas yield and gas composition occurs.

The pH value is also measured on site by instructed operators using common pH measuring instruments. The value is only suitable to a limited extent for an accurate process assessment; the buffer properties of the fermentation mixture cause a sluggish reaction of the pH value (Lindner et al., 2015). Nevertheless, the pH value is an important value to record. The recording of the measured values shows tendencies and trends, which can be used to draw conclusions about other parameters such as ammonium and ammonia. Prolonged standing or sample transport changes the pH value of the sample; on-site pH values are usually somewhat lower than those determined in the external laboratory.

The biological processes are strongly dependent on the pH value, for optimal methane formation it lies in a range between 7 and 7.5, formation of biogas is also possible outside this window. In a single-stage system, the pH value automatically levels off; the bacterial groups act here as a self-regulating system. In multi-stage systems, the pH value at the hydrolysis stage is between 5 and 6.5, as the acid bacteria are at their optimum here. In the methane-forming phase, it is again in the neutral range, due to the buffer capacity of the medium and the degradation activities. By means of the pH value, the dissociation equilibria of metabolic products such as ammonia, organic acids and hydrogen sulphide can be controlled. Because of the buffering capacity of the medium (hydrogen carbonate and ammonium), the pH value remains stable. If the pH moves outside the optimum, this is an indication of a serious process disturbance, immediate action should be taken. Volatile fatty acids are an intermediate

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product in fermentation. At high concentrations, they have a process-inhibiting effect. If the individual acids are analysed, this can be used to describe the state of the process. Acetic acid should ideally be present in the low single-digit gram range per litre. The concentration of propionic acid should be well below this, and other fatty acids should be lower by a power of ten. Individual fatty acids are determined chromatographically. The acids dissociate as a function of pH in aqueous solution. The fatty acids investigated are acetic acid, propionic acid, iso-butyric, butyric, iso-valeric, valeric and carboxylic acids (Fachagentur Nachwachsende Rohstoffe, 2010b). The individual organic acids are expressed in mg/l, and the acetic acid equivalent is also calculated. This is a sum parameter; the different weights of the fatty acids are uniformly calculated to the weight of the acetic acid. The acetic acid equivalent and the propionic acid are the indicators used for process evaluation. If the total acid value is less than 1 g/l and the propionic acid content is less than 200 mg/l, the process is stable. If the values exceed 3 g/l and 300 mg/l for propionic acid, the degradation process is not optimal and there is a malfunction (Fachagentur Nachwachsende Rohstoffe, 2010b). In case of intermittent feeding with large quantities the acid concentration increases, if the same total quantity is distributed over several smaller doses the acid concentration remains low. If the load is too high due to incorrect feeding, not all long-chain fatty acids can be broken down. This leads to an accumulation of propionic and butyric acid. In the case of overfeeding or fluctuating substrate contents, an accumulation of acetic acid occurs, this is associated with a decrease in methane content. The non-degradation of acetic acid is associated with a decrease in the activity of methanogenic bacteria.

2.3 Process variants

2.3.1 Dry matter content

For substrate characterization, the amount, concentration, and composition of the substrate must be known. For the concentration, parameters such as dry substance content (DM) and organic dry substance content (oDM) are specified. For liquid substrates, the chemical oxygen demand (COD) parameter is also added, and another parameter is the total organic carbon (TOC) value. For practical purposes, only the DM and the oDM value are relevant. The oDM degradation rate indicates how much of the added organic DM was degraded during the fermentation process.

When determining the DM value, a sample of the substrate to be fed in is taken. The DM value of substrates is usually measured by operators on site. Often, several substrates with different DM content are used; the DM content of the substrate mixture is decisive and not the DM content of the individual substrates (Streicher et al., 2016).

To determine the oDM value, the sample is calcined at 500 °C. The loss of mass is called organic mass loss. The loss of mass is called organic dry matter. The oDM parameter is not very meaningful in terms of degradability or expected biogas production. When the sample is heated, volatile substances are evaporated and are therefore no longer available for analysis. This leads to deviations in the estimation of the gas potential, especially in the case of

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highly acidified substrates. A residue is left behind when the gas is burned up; this represents the proportion of inert ingredients in the substrate (Fachagentur Nachwachsende Rohstoffe, 2010a). The oDM content cannot be determined on site and is therefore less used by operators to assess the substrate.

Process differentiation based on DM content: From a biological point of view, a strict division of the processes into wet and dry fermentation is misleading; the microorganisms require a liquid medium for their growth and survival in any case. There is no precise definition of the boundary between wet and solid-state fermentation, but guide values have become established in practice. A DM content of up to approx. 12 % in the fermenter is referred to as wet fermentation; the fermenter contents are still pumpable under these conditions. If the DM content increases to 15-16 %, this process is referred to as solid matter fermentation or dry fermentation, and the material is generally no longer pumpable (Streicher et al., 2016). The rule of thumb is not always applicable, there are some substrates (e.g., dispersed food waste or poultry manure) with finely dispersed particle distribution, which are still pumpable at a DM content of up to 20 %. Other substrates, such as fruit peels, are in stackable form at a DM content of 10 % (Lossie and Pütz, 2021). For stable process control, the DM content of the substrate used should remain constant; if it increases too much, the substrates must be further liquefied; if the DM content falls, it must be counteracted with appropriate substrates with a high DM content. The higher the DM content, the higher the gas production. **Table 16** shows an overview of guide values for mass decomposition and DM content after fermentation for different individual substrates. In any case, however, the agitation and conveying technology must be adapted to the DM content in the fermenter.

Table 16: Guide values for mass degradation and total solid content after fermentation for different individual substrates (Reinhold, accessed 04.11.2021).

	Unit	Cattle slurry	Pig slurry	Stable manure	Dry chicken manure	Corn silage	Corn-Cob-Mix	Grain
DM	%	8	6	25	45	32	65	86
VS	%	80	80	80	75	95	95	98
Mass reduction	%	2.3	2.3	11	19	24	55	75
VS reduction	%	33	46	52	58	70	79	81
DM-Content after Fermentation	%	5.9	3.8	14.5	25.4	10.8	14.7	17.4

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2.3.2 Number of process stages

One stage refers to the process-specific biological environment - hydrolysis or methanation phase (Lossie and Pütz, 2021). If the fermentation process takes place in one system at one temperature and pH under identical process conditions, this is a single-stage process. If two fermentation tanks are used with separate hydrolysis and methanation, this is two-stage process control. The phase denotes the process tank independent of the biological stage (Nasr et al., 2012). In a single-stage system, there is no spatial separation of the phases (hydrolysis, acidification phase, acetic acid formation and methane formation). For two-stage or multi-stage processes, spatial separation of the phases is aimed at different vessels. For two-stage processes, the hydrolysis and acidification phases take place in separate external vessels. A two-phase or three-stage biogas plant consists, for example, of a hydrolysis tank, fermenter, and secondary fermenter.

In the case of continuous processes, there are three relevant variants. A gas-tight sealed receiver is not considered as an independent phase (Lossie and Pütz, 2021).

1. flow-through process (see **Figure 42**): downstream of the digester is an open digestate storage tank. This variant is two-phase because different temperatures prevail in the preliminary pit and the fermenter. The open digestate storage does not count as fermentation, since gas formation should already be completed in the fermenter to allow open storage.
2. storage process: The digestate storage system also serves as a post-digester to increase the retention time in the gas-tight system. This variant is three-phase and two-stage.
3. combined flow-through storage process (see **Figure 42 & 43**): this combination consists of a digester and a gas-tight, covered digestate storage. Since the digestate storage still captures biogas, this is considered another stage.

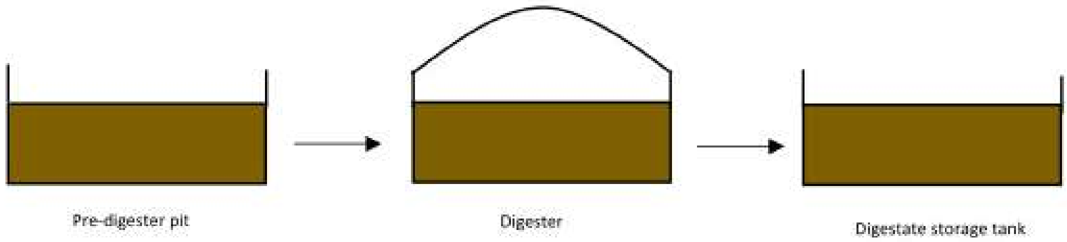

Figure 42: Schematic of the through-flow process (two-phase, two-stage) (Fachagentur Nachwachsende Rohstoffe, 2010b).

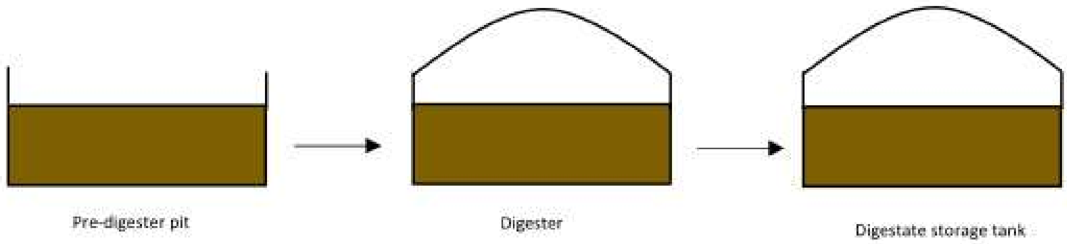

Figure 43: Schematic of the combination throughflow (two-phase, three-stage) (Fachagentur Nachwachsende Rohstoffe, 2010b).

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Flow-through method: In the past, most plants were operated according to the flow-through method. The substrate is pumped or conveyed from a storage tank into the fermenter. The identical quantity that is fed to the fermenter in this process enters the digestate storage via displacement or withdrawal. The digester is permanently filled and is only emptied if required for maintenance tasks. This process has good and uniform gas production, as well as good digester utilization. Short-circuit flow can occur under unfavourable conditions, but this is usually prevented by the arrangement of feed and discharge. There is a risk of methane emissions in an open digestate storage system.

Combined flow-through and storage process: In this process, the digestate storage is also covered. Biogas is produced in the digestate storage facility, which can be collected and utilized. The digestate storage system acts as a storage facility for gas and digestate. The storage facility is downstream of a flow-through fermenter. This process allows uniform gas production and is state of the art.

For all three processes, regular feeding must take place in small quantities. This applies in particular to single-phase operation without hydrolysis stage. Intermittent feeding leads to an overload of the fermenter biology. Multi-stage operation allows material to be recycled from the secondary digester to the digester. This may be necessary should the methane fermentation phase be additionally stabilized (Lossie and Pütz, 2021).

Discontinuous feeding: Discontinuous processes are usually operated in two stages. The two stages consist of a box fermenter and percolate storage. A fermenter battery usually consists of four units, whereby quasi-continuous operation is achieved. While one digester is being fed, the remaining digester units are in operation. To feed a fermenter, the percolate regime is set beforehand (Nasr et al., 2012*)*.

Discontinuous processes can be divided into single-phase and two-phase variants.

1. Solid-state fermentation single-phase: the substrate in the box unit is inoculated and strongly percolated so that it enters the methane phase as quickly as possible. The methane phase is permanently given in the percolate storage (Lossie and Pütz, 2021*)*.
2. Two-phase solid matter fermentation: Here, there is no inoculation of the fermented material. As a result, mainly hydrolysis/acidification is created in the pit fermenter. The percolate reservoir thus represents the only methane phase.

2.3.3 Temperature control

In practice, there are two main temperature ranges: mesophilic and thermophilic. In practice, these are operated at temperatures of 36 °C to 44 °C (mesophilic) and 50 °C to 58 °C (thermophilic). **Figure 44** shows the relative gas production as a function of the fermenter temperature of mesophilic and thermophilic bacterial strains. The optimum temperature for many bacterial strains involved in the fermentation process is between 30 - 40 °C. In this temperature range there is a higher gas production, a higher species diversity and lower

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concentrations of inhibitory ammonia. The thermophilic temperature range is above the optimum for hydrolysis and acidogenesis and is an optimum for aceto- and methanogenesis. If harmful germs are to be killed, the thermophilic mode should be used. Compared to the mesophilic mode, the thermophilic mode has a better biogas yield, but only a slightly better methane yield can be achieved. Thermophilic operation is more susceptible to failure because it is subject to instabilities. This mode of operation has a lower number of different microorganisms compared to mesophilic operation. The thermophilic mode is more susceptible to temperature fluctuations, especially when the temperature is lowered. However, the higher the temperature, the faster the substrate turnover occurs only within certain limits, as other factors limit. **Table 17** shows the temperature control in a multistage operation. Which combination is used, depends mostly on the substrate composition. The most used combination is mesophilic/mesophilic, which is used for slurry and most raw plant materials. Components with thermophilic operation are used for substrates with increased fibre content, such as green waste or vegetable waste (Chae et al., 2008*)*.

Table 17: *Overview of temperature control for multistage operation (Postel et al., 2009).*

Fermenter	Post fermenter	Advantages	Disadvantages
mesophilic	mesophilic	stable biology	Organic degradation takes longer
thermophilic	mesophilic	fast structure decomposition, better flowability in further process chain	Biology susceptible, as fermenter biology most sensitive
mesophilic	thermophilic	stable biology with slightly increased gas yield and good flowability from secondary fermenter	Increased ammonia content in the biogas stream puts a strain on the CHP engine (see below)
thermophilic	thermophilic	Increased gas yield (but with somewhat lower methane content)	Vulnerable operation, usually less energy available for external CHP use.

The secondary fermenters or digestate stores are usually not heated, but secondary fermenters are often equipped with thermal insulation. Thermophilic operation increases the proportion of trace gases such as H_2S and N compounds in the biogas stream. As the temperature increases, the NH_3-NH_4-N equilibrium shifts toward ammonia. Mesophilic operation results in less organic degradation for the same residence time; this can be compensated by longer residence times.

The thermophilic mode of operation reduces the risk of technical failure, this is due to the lower viscosity of the digestate. This protects the pumps used as well as the valves. However, this is only the case if all plant components involved are appropriately designed for higher temperatures. However, there is an increased risk of foam formation, which can escape via the safety equipment.

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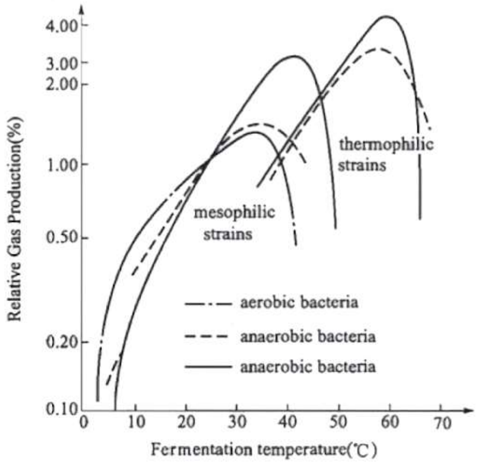

Figure 44: Fermenter temperature vs the relative gas production rate.

2.3.4 Auxiliary materials/additives

Normally, relatively monotonous substrate is used in biogas plants when using energy crops, which can lead to deficiency symptoms as well as to the accumulation of inhibitors. A mix of different energy crops and the use of manure can minimize the need for the use of fermentation aids. The use of fermentation additives depends on the substrate used, fermentation conditions, and other factors. Additives are substances added to the fermentation process with the aim of positively influencing it. All substances or working materials added to the fermenter to promote the microbiological degradation processes, which are not substrates, are auxiliary substances. The fermentation aid itself has no biogas formation potential or this is negligible. Fermentation aids can be of organic or inorganic composition (including algae preparations, trace elements for supplying the microorganisms, enzymes for hydrolysis) (*Messtechnik für die Biogasanlage…*, accessed 10.11.2021).

Two applications can be defined for the use of fermentation aids, on the one hand the one-time addition in the event of a malfunction and on the other hand the prophylactic-preventive application. Practical experience has shown that instabilities in the fermentation process can occur if liquid manure and solid manure are not used. This can be caused by a lack of micronutrients as well as the presence of inhibitors. In practice, this is counteracted by using fermentation aids. There are different reasons for using them, e.g., the specific methane yield is too low [m^3/kg_{oDM}], failure to achieve higher digester load [$kg_{oDM}/(m^3 * d)$], reduction of hydrogen sulphide, high viscosities and insufficient mixing, foaming and floating layer formation. The examples listed are indicative of a process upset. Prompt laboratory analysis allows the operator to intervene quickly and efficiently. The symptoms can be traced back, for example, to an accumulation of volatile organic acids, a shift in the acid spectrum towards higher-value fatty acids, an increasing FOS/TAC value, an increased concentration of ammonia. Also, causal factors can be an insufficient degradation efficiency as well as an increasing solids content in the fermenter (*Messtechnik für die Biogasanlage…*, accessed 10.11.2021).

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These examples illustrate that, in addition to the efficiency of the biogas plant (gas yield with short retention time), the ongoing operating costs are also a reason for using auxiliary materials. These can be used to reduce the energy required for the agitators, and the cost of desulfurization can also be reduced. If the benefit of fermentation aids exceeds the expense, their use is recommended. **Table 18** shows typical fermentation aids with examples and the areas of application. A clear subdivision of the individual substances is difficult in that they often have several modes of action at the same time.

Legal regulations apply to the use of auxiliary substances; too high an addition of these can lead to the fermentation residue no longer being allowed to be discharged, for example.

Table 18: Typical fermentation aids with examples and areas of application (Fachagentur Nachwachsende Rohstoffe, 2010b).

Type of fermentation additive	Examples	Definition
Trace elements	Iron, Cobalt, Nickel, Zink, etc.	Trace elements are chemical elements, that are necessary for the optimal growth of microorganisms.
Ion exchanger	Zeolites, Clay minerals	Reduce the concentration of potentially inhibitory/toxic fermenter ingredients (also in relation to further treatment)
Microorganisms	hydrolytic cultures	Completion of the existing biocenosis Organisms that optimize the process (Speed, stability) or faster adaptation to new substrate compositions or changed Enable boundary conditions
Enzymes	Cellulase, Amylase, Protease, Xylanase	Enzymes break down polymers and improve suspension properties, increase the degradation rate and support microbial activity.

Trace elements: The microorganisms involved in anaerobic fermentation require nutrients, the so-called bulk elements, to maintain metabolism and for their own reproduction. These bulk elements are: Hydrogen (H), Carbon (C), Nitrogen (N), Oxygen (O), Phosphorus (P) and Sulphur (S) (12). Also, sufficient sodium (Na), potassium (K), calcium (Ca), iron (Fe) and magnesium (Mg) must be present (Drosg, 2013). To maintain their metabolism and to produce enzymes, microorganisms need so-called trace elements. These are involved in enzymatic conversions, in the formation of co-factors, in redox reactions and other processes in which living organisms are involved. Trace elements in the biogas reactor include nickel (Ni), cobalt (Co), molybdenum (Mo) and selenium (Se). But also, other metals, such as copper (Cu), zinc (Zn), manganese (Mn), tungsten (W), or vanadium (V) and nonmetals such as boron (B)

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(Drosg, 2013). If a plant is operated with slurry or manure, enough trace elements are available to the biogas plant, but a deficiency can still not be excluded. Biogas plants that use only corn silage have long-term trace nutrient deficiencies. These deficiency situations occur with mono fermentation of energy crops. Trace elements usually come from the substrate, but are often not available in required amounts, so the additional supply of trace elements is the rule in practice. Biogas plants with a very high slurry input, good management and low space loading can do completely without the supplementation of micronutrients. Trace element supply is a function of the biocenosis in the digester and is determined by technical, physical and chemical conditions. Poorly soluble precipitated sulphides in the fermenter, for example, can reduce the concentration of trace elements by several orders of magnitude. Agitators can release trace elements, which leads to an increase in the concentration of trace elements. The trace element contents added must be determined by an external laboratory service provider and must be repeatedly validated if the process is stable. Proper use of trace elements can accelerate fermentation, results in a more stable process, and greater space loads and biogas yields can be achieved. Overdosing can have an inhibitory effect on the fermentation process; too many elements have a toxic effect on the biocenosis (Drosg, 2013).

Enzymes: These are proteins that are involved in degradation processes as biological catalysts. In normal operation, these enzymes are produced by the microorganisms; with an optimal fermenter operation, the new enzyme formation is ensured. According to the manufacturer, the addition of enzymes improves the degradability of plant components, which should be broken down more quickly. This is said to improve the productivity of the gas process. By adding enzymes, a faster and more intensive digestion of the biomass is to be achieved, the viscosity is to be reduced, which lowers the stirring energy costs. Furthermore, a faster degradation of cellulose and hemicellulose is to be achieved, floating layers in the fermenter are dissolved. Massive piles of solids from grain, hay, grass silage and solid manure are to be better dissolved and liquefied, floating layers in the final storage are to be avoided with enzymes. Practical studies had shown that there can be significant effects with substrates rich in water and through the application of enzymes. Enzymes do not have a protein structure and are therefore subject to bacterial degradation; for a lasting effect, appropriate preparations must be continuously added to the process. (Bochmann et al., 2007).

Additives to reduce the hydrogen sulphide concentration: Gaseous hydrogen sulphide is formed in the biogas process from organic compounds of sulphur, which are reduced to hydrogen sulphide by microorganisms. Increased levels of organic sulphur components are present in canola and rapeseed products and in protein-rich substrates such as grain, food waste, pig manure, and poultry manure. Hydrogen sulphide affects many microorganisms and can inhibit methane formation in the digester. Sulphide precipitation binds trace elements in the fermenter, which are thus no longer available to the microorganisms. By using the auxiliary substances iron (II) and iron (III) salts such as $FeCl_2$, $FeSO_4$, $FeCl_3$ and $Fe(OH)_3$, a targeted precipitation of sulphide is possible so that it does not enter the biogas as hydrogen sulphide (Meegoda et al., 2018).

Additives for the reduction of the ammonia concentration: Ammonia occurs in low concentrations in biogas. It is formed during the breakdown of proteins and nitrogenous com-

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pounds such as urea or uric acid in animal excrement. To reduce the ammonia concentration, mineral substances are added as additives. These bind excess ammonium ions. Molecular sieves and clay minerals, which can fix ammonia by ionic forces, are also used for the sorption of ammonia. In this process, cations such as potassium, calcium or magnesium are replaced from the lattice structure and bound to ammonium as in an ion exchanger. The ammonium remains bound to the solids and is thus removed from the system. If these additives are used incorrectly or too frequently, sink layers may form, the effectively usable fermenter volume decreases as a result, and there may also be an abrasive effect on the agitators (Drosg, 2013).

Minerals and buffers (pH stabilizers): Methane formation requires a stable pH value. The pH value is influenced by the base and carbonate concentration or the lime-carbonic acid balance, as well as by the ratio of ammonium to ammonia. If excessive amounts of carbonic acids are formed in the fermentation process, acidification of the fermenter contents may occur. To counteract this, buffering substances such as sodium hydrogen carbonate or combination preparations with trace elements and carbonates can be used. These are intended to safeguard the pH value against over acidification (Drosg, 2013).

Floating layer remover: Floating layers (see **Figure 45**) can form on the surface of the liquid in fermenters, secondary fermenters or in the digestate store. Floating layers are foamy, dense to solid deposits that may result from lack of mixing, incomplete decomposition of the organic mass, high space loading or hydraulic short circuits. Using enzymes and algae extracts, etc., long-fibered substrates can be better degraded. The hydrolytic degradation should be faster and the viscosity should be reduced, which leads to an improvement of the stirring performance. As a result, floating layers can be dissolved more easily (Drosg, 2013).

Defoamer: One of the most common malfunctions in biogas plants is the formation of foam. It can lead to blockage/clogging of the pipelines and thus to severe damage to the plant. Pipelines can become fouled, sensors can be disturbed, and gas lines can become clogged. In the worst case, tank leaks can occur. Foam is an important indication that the operation of the biogas plant is faulty. Foams occur when substrates with a high protein content, such as grain, potatoes or dry poultry manure, are fermented. Fats can be released, which are hydrophilic and collect at the liquid surface. There they stabilize formed foam by binding hydrophobic membranes around the air bubbles. Increased acid formation within the fermenter can also contribute to foam formation. Natural oils such as rapeseed oil and silicone oils as well as poly alcohols are used as defoamers. These affect the surface tension of the liquid and change the properties of the bubble formation. In the case of foam formation, the contents of the fermenter are insufficiently stirred and the feedstock and nutrient exchange at the surfaces of the fermentation mixture is reduced. This results in a lack of nutrient supply to the microorganisms, and their cell density and growth rate are negatively affected. The physical gas discharge from the liquid medium is also impaired, which can lead to inhibition or process breakdown (Drosg, 2013).

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Figure 45: Large foam bubbles in the fermenter formed by the use of sugar beet (Kliche and Lebuhn, 2017).

Microorganisms: The addition of microorganisms is intended to accelerate biogas production, increase degradation and improve gas yield. The load capacity of the biogas plant, or more precisely the space load, is to be increased with high-performance bacteria (Drosg, 2013). The addition of special externally bred species also has its drawbacks. These have to show a high performance and face the selection pressure within the biocenosis. However, they can displace existing species in the process. If the species do not grow naturally on a sufficient scale, they must be added again and again (*Messtechnik für die Biogasanlage...*, accessed 10.11.2021).

Capillary carbon: Plant carbon is not to be understood here as a fertilizer, it is a carrier substance and serves the periodic storage of essential nutrients. It provides habitats for microorganisms. Two types of charcoal can be distinguished, one is charcoal with a large surface area, which favours the growth of bacteria. On the other hand, charcoal with a high capillary density, which particularly ensures effective material flows and substrate supply (Franke, accessed 10.11.2021). Capillary coal consists to a large extent of pure carbon and thus forms an ideal habitat for microorganisms, which can live and multiply there. The pH value of the charcoal is between 8 - 8.7 and thus provides an ideal growth habitat for methane-producing archaea. The structure of the coal ensures pressures in the pores and capillaries, which guarantee the substrate supply for the microorganisms. Salts can be introduced into the pore structure and serve as nutrients. In the acetogenic phase, the carbon suppresses the sulphate-reducing bacteria and promotes the denitrifying bacteria. This promotes the formation of acetic acid, which generates a higher methane yield (Franke, accessed 10.11.2021).

2.4 Automation and control technology

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2.4.1 Sensors

In practice, the process of biogas production is focused on a few essential parameters in order to reduce the effort of evaluation as well as the acquisition and maintenance costs. Some values such as filling levels, gas composition or power consumption and power generation are recorded online and evaluated without loss of time. Other parameters such as the FOS/TAC value, DM and oDM values are recorded discontinuously, and in some cases determined and evaluated in external laboratories. Values determined off-line and in external laboratories are prone to errors; inaccuracies occur due to sample collection and transport, making precise and timely process control difficult (*Anleitung zur Ermittlung des FOS/TACs*, accessed 03.11.2021). Sensors must often be used in areas where explosive gas mixtures may occur. These sensors must then be suitable for this and must also be installed accordingly. In addition, the sensors must be resistant to high humidity and corrosion. Measuring devices with moving parts in the biogas stream are susceptible to malfunctions due to contamination. Special requirements also apply to all sensors that must trigger safety shutdowns. For example, level sensors that must shut down the filling of the tank when a maximum level is reached. Depending on local regulations, these probes must be connected by cable and trigger shutdowns via relays. Other sensors can communicate directly with the plant control system via BUS systems. Some important process control parameters and their measurement are explained below.

Input quantity and substrate composition: For solids, it is advisable to weigh them, for example by means of scales embedded in the floor on which, for example, loaded machines are weighed before and after unloading. Pressure sensors are used for feeding systems such as the solids feeder. Flow measuring devices can be used on the pipelines for the introduction of liquid substrates, and the volume added can also be determined using level measuring devices. Inductive and capacitive sensors are mainly used for flow measurements, ultrasonic and thermal conductivity sensors are also used less frequently.

DM measurements in the fermenter with microwave spectroscopy (Figure 46): Meanwhile, there are compact and robust microwave-based measuring systems that have been developed specifically for determining the DM content in biogas plants. These can record the individual or total DM content of the substrates involved. There are also versions that are specifically designed for digester installation. Usually mounted in a guard, the sensor measures through a Plexiglas front and there is no direct contact with the fermentation substrate. Microwave radiation passes through a dielectric window of its shroud and detects a representative sample volume. Solids can be detected in water because their relative dielectric constant is much lower (about 2 to 10) than that of water (about 80). Reflective microwave arrays are used for DM measurements. An electromagnetic wave with very low energy is radiated into the material from an antenna and reflected. The reflected wave is detected again and the reflected portion depends on the dielectric properties of the material under investigation. This provides information on the water or dry substance content (Henkelmann et al., 2010). The method is not very susceptible to interference, changes in pH values or conductivity have no effect, the microwave DM sensors are available in a wide variety of designs, can be retrofitted and have long-term stability.

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Figure 46: Microwave DM sensor MWDM PP tube in a waste digestion plant (Göller, accessed 2021).

Mobile DM measurement: This measurement is performed gravimetrically. The measuring device has a heated balance for this purpose. A sample of the material to be measured is weighed in the moist state and then automatically dried until the weight is constant. The water content is calculated from the difference in mass. This method cannot be operated continuously but has the advantage that the device is inexpensive and easy to operate and can also measure substrates and fermentation residues. These values are also important for the operation management.

Level measurements: To detect the amount of energy present in the gas storage tank, the pressure, temperature, gas composition and storage volume must be known. A variety of sensor types exist for level detection in the fermenter, these can be hydrostatic pressure sensors, also distance measurements to the surface can be determined by ultrasound or radar.

Way rope sensors (Figure 47) are sensors for length, displacement, and position determination. Mechanical level gauges are often used in double diaphragm accumulators to determine the height of the inner gas diaphragm. The filling level of the gas accumulator can be determined by the distance between the gas accumulator diaphragm and the weather protection diaphragm. Draw-wire sensors attached to the gas storage membrane are used for this purpose. The cable is guided through the storage membrane and ends in a measuring

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tube via deflection pulleys. If the diaphragm lifts, the change in the sensor's travel is registered. Magnets are installed at the end of the cable, and these switch via installed reed contacts in the measuring tube. The output signal, which is proportional to the displacement, is converted into a level. This technology is simple but relatively robust and reliable.

Figure 47: Ropeway with sensors.

Rod probes, measure the electrical conductivity between metal rods. Depending on the number of bars installed, different measuring points can be detected. They are often used for leakage detection and shutdown in case of overfilling. Leakage probes are used to detect leaks in tanks containing liquids hazardous to water. On contact with an electrically conductive liquid, the integrated electronics react, and a permanently emitted signal is interrupted. This can result in an acoustic or optical signal. Overfill sensors are mostly self-sufficient and not coupled in a bus system. Rod probes can only determine discrete levels. Other systems are used for continuous measurement.

Pressure probes can continuously and reliably measure liquid levels. For this purpose, the probe is installed near the bottom of the tank. This technology is widely used to determine the levels in tanks of biogas plants.

Radar sensors are also suitable for continuous level measurement. The advantage of radar sensors is their maintenance-free operation due to non-contact measuring methods and the fact that they provide exact measurement results independent of the process conditions. The instrument emits a continuous radar signal via its antenna. The signal is reflected by the medium and received by the antenna as an echo. The frequency difference between the transmitted signal and the received signal is proportional to the distance. This depends on the level and can therefore be determined. Radar probes can be used more flexibly than pressure probes. They can also detect foam formation.

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Figure 48: Radar measuring device for level measurement (Micropilot FMR52 from Endress+Hauser) (Messtechnik für die Biogasanlage..., accessed 10.11.2021).

Ultrasonic measurements based on the transit time principle offer another non-contact method. A sensor emits ultrasonic pulses, they are reflected by the surface and detected by the sensor. The required transit time is a measurement of the distance travelled in the empty part of the tank (Arbeitsgemeinschaft Landtechnik, accessed 08.11.2021). This value must be subtracted from the total height of the tank, from this the level can be calculated. Radar uses electromagnetic waves, while ultrasound uses mechanical waves that propagate at the speed of sound. Ultrasonic level sensors are insensitive to changes in density and viscosity. Foam, turbulence, vapor and haze can affect measurements. Other optical sensors such as laser measurement have problems with steam and particles and are therefore not generally recommended.

Biogas production: A change in gas composition or gas volume can be an indicator of process imbalance. Accurate measurement of biogas volume is a technical challenge because biogas is composed of different gases, is saturated with water vapor, and may also contain particles. In addition, biogas is produced at low pressure. Gas metering systems should be located so that they are easy to remove and clean. There are many different types of sensors available for gas meters, these and their typical advantages and disadvantages are listed in Table (*Arbeitsgemeinschaft Landtechnik*, accessed 08.11.2021). Flow sensors are used for both gas flow and substrate flow. Special requirements apply if the determined values must be used for billing purposes. When biomethane is fed into the natural gas grid, the meters are provided by the grid operator.

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Table 19: Overview of advantages and disadvantages of different sensors for gas volume measurement (Drosg, 2013).

Sensor Type	+	-
Ultrasonic flow meters	• Good results at low pressure • No moving parts • Very reliable even at changing process conditions	• Long straight measuring distance needed (15 times the diameter)
Fluidistor oscillator	• No moving parts • High accuracy • Low cost • Easy handling, exchange and cleaning	• Complex calculation to norm cubic meters • Error of 1.5% • Sensitive to vibrations in the biogas caused by e.g., piston compressors
Turbine flow meters	• Robust technology	• Deposits can become problematic • Moving parts
Vortex flow meter	• No moving parts • High durability • Resistant to corrosion • Low pressure loss	• Sensitive to disturbances in flow • Long straight measuring distance needed (30 times the diameter)
Dynamic pressure probes	• Long durability • Dirty gas has little influence • Pressure fluctuations have no negative effect on accuracy	• Works better at higher gas pressure • Large calibration effort • Error of 1.5-5% • For calculation of Nm^3 the density (gas composition) is needed • Long measuring distance needed
Thermal flow meters	• Easy handling • Good for mobile applications • Direct measurement of Nm^3/mass • Exact Measurement also at pressure fluctuations	• No dirty biogas measurement possible • Measurement error of 3-5% (increases rapidly if gas is dirty) • Extremely sensitive to humidity • Long straight measuring distance needed • Calibration once a year
Diaphragm gas meters / bellows gas meters	• Simple and cheap • Direct volume measurement • Robust technology	• Corrosion, fouling or deterioration of gas meter by biogas components and particles • Increased utilization time decreases accuracy of measurements • External calibration and maintenance

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Gas composition: In many biogas plants, online measuring devices are installed to record the gas composition, but portable measuring devices are also used. **Figure 49** shows a stationary analysis station from Binder Engineering GmbH. It detects CH_4 and CO_2, which are measured with infrared or thermal conductivity sensors. In the vast majority of cases, H_2S and O_2 are determined by electrochemical sensors. Oxygen is also measured by paramagnetic sensors. H_2S is measured less frequently than other parameters, this increases the lifetime of the sensors. Measurement of biogas composition is not necessary for modern CHP units, they are self-regulating, however, biogas composition is a useful parameter to monitor the processes in a biogas plant. For example, a decrease in methane content may indicate an overload, and an increase in H_2S may indicate process instability. The table shows different measurement methods or gas sensors for the analysis of biogas. Methane, carbon dioxide and oxygen are indicated in vol.-%, hydrogen sulphide is usually indicated in ppm.

Figure 49: Flexible modular gas analysis station (COMBIMASS® GA-s hybrid premium; Binder Engineering GmbH) (Binder Engineering GmbH, accessed 09.11.2021).

Table 20: Suitable gas sensors for the analysis of biogas (Franke, accessed 10.11.2021).

Measurement method	CH_4	CO_2	O_2	H_2S
Thermal conductivity sensor	X	X		
Spectrometer valence electrons (Near Infrared)	X	X		
Spectrometer valence electrons (UV light)				X
Spectrometer Molecular Vibrations	X	X		
Electrochemical gas sensors			X	X
Paramagnetic sensor			X	

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Temperature: Biogas digesters require a stable process temperature; this is necessary for high performance of the microorganisms. Pt100 T probes are mainly used to measure the temperature. They must cover a temperature range of 20 - 60 °C to be able to measure in the psychrophilic, mesophilic, and thermophilic fermentation environment. Due to measurement inaccuracies, it is recommended to use several temperature sensors in different local areas. Pt-100 measurement sensors are very accurate temperature sensors, all parts are made of stainless steel, they are characterized by their robust mechanical design and low cost.

Electricity meter/power feed: An electricity meter is a measuring device that records the energy supplied and the instantaneous power of a consumer. The measured values are given in kilowatt-hours for energy and in kW for power. An electricity meter in a biogas plant evaluates not only the kilowatt-hours delivered to the grid operator, but also the own consumption and the most important electrical consumers of a biogas plant are the CHP, agitators, solids input, solids and slurry pumps, drives, the support air blowers, and pumps. The average own power consumption is between 6 - 12 % of the generated electrical energy. The energy consumption of aggregates can be measured at various points and is evaluated by the plant control system.

2.4.2 Power feed-in

Grid connection point and transfer station: The electrical energy generated in the CHP unit can be fed into the low-voltage grid (usually 400 V) or medium-voltage grid (7 - 25 kV (after conversion in the transformer)). The power is fed into the grid at the grid connection point, where the operator's power grid merges with that of the power supplier. At the point of connection to the grid, the energy fed in and taken out must be measured and protective devices must be installed for disconnection in the event of a fault. These installations form the transfer station.

Telecontrol technology, control specifications of the grid provider: In most cases, the transfer station must be included in the communication of the plant control system since the grid provider must be able to throttle the power of the plant in case of emergency or to take it off the grid completely. In simple cases, the transfer station contains a radio receiver for this purpose, which acts on the CHP unit through a direct wired connection and regulates it in stages (30%, 60%, 100%). The connection to the CHP unit is then made via a switching contact without the system controller being interposed. This ensures that the control from the grid provider always has priority over other controls. In new buildings, the grid provider usually communicates with the plant control system and the CHP unit via a BUS system. The advantage is that the grid provider receives important information for grid operation, such as available power or available runtime (for flexibly operating plants). Disadvantage is a considerable financial expenditure for the installation of this remote-control technology. The grid provider determines which method is used. Regardless of the grid provider's ability to

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intervene in power generation via remote access, general compliance with various characteristic curves is often required by the grid provider. Such characteristics can, for example, control the reactive power fed into the grid as a function of the power or the grid voltage. Depending on the control concept, such characteristic curves can be stored directly in the CHP controls or in the plant control system.

Protection concept: During the planning phase of the power feed-in, a protection concept must also be drawn up. It regulates the separation of the generating unit (CHP) and the power grid from the grid of the generating plant in the event of a fault (overcurrent, undervoltage and overvoltage, frequency protection, shutdown if the contractually regulated power is exceeded, shutdown if the reactive power is too high, etc.). Basically, the protection takes place in two stages. The higher-level protection disconnects the entire plant from the power grid (this usually also means a power failure on the entire plant). The subordinate protection disconnects only the generating unit from the mains. The protection functions must be parameterized in such a way that the subordinate protection trips first.

2.4.3 Plant control

The plant control system handles the communication between the individual plant components, aggregates, and their control. It essentially consists of electrical components such as frequency converters for speed-controlled motors, relays for switching and the programmable logic controller (PLC) on which the programming is stored. Usually, the PLC has a visualization of the plant on which the most important parameters can be read and many values can be set.

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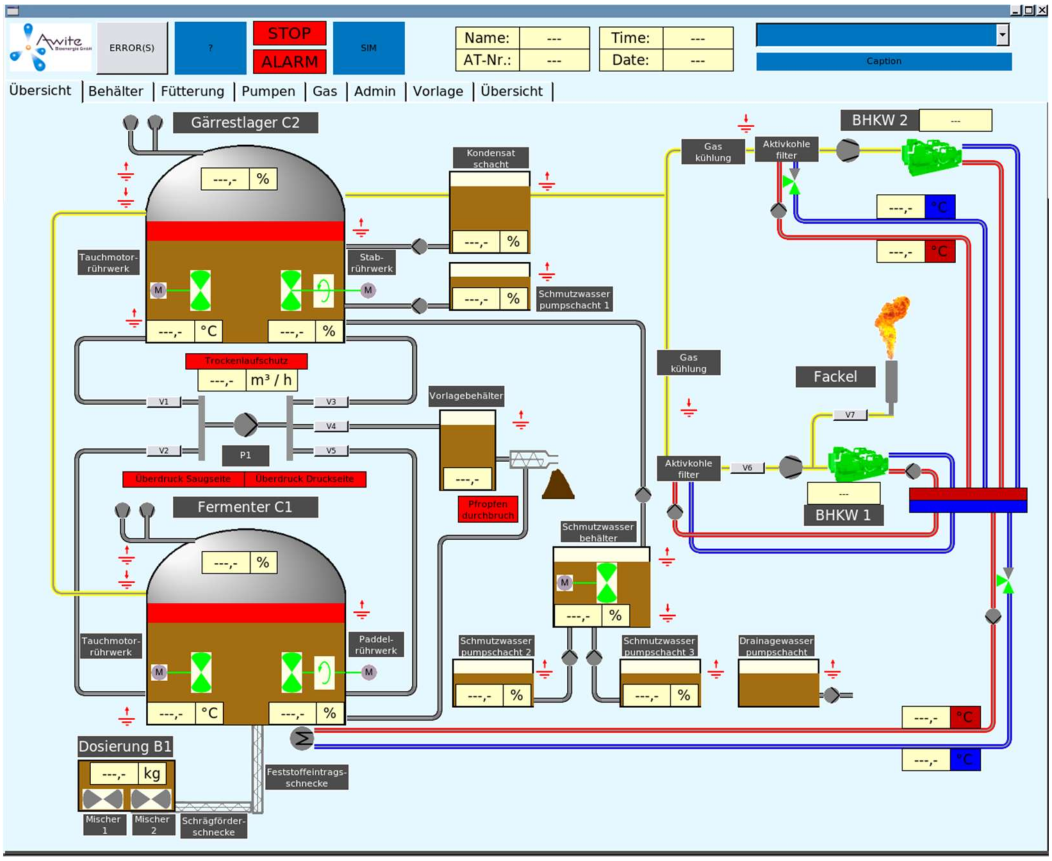

Figure 50: Example of a plant visualization (AEV Energy).

The basis of the control is the programming of the PLC. Here, the running times of the stirrers, the feeding quantities of the tanks, the running times, and the schedule of the CHP and much more are set. In the event of malfunctions, for example the failure of a pump, the PLC must register this and issue an error message. Usually, this error message is also forwarded as a text or voice message to stored telephone numbers. In addition to the control tasks required to maintain operation, the system controller must also perform safety shutdowns. A special feature here is that these switches do not run via the PLC, but the corresponding relays are controlled directly. This concerns, for example, the starting of the gas flare when the maximum gas storage level is reached or the switching off or switching on of pumps when min. or max. filling levels are reached. This is intended to increase process reliability.

2.4.4 Emergency power supply

Prolonged power failures can lead to process disruptions and dangerous situations. Therefore, various aggregates must be able to be supplied with emergency power within a short time. As a rule, emergency power generators are used here which are started manually or automatically in the event of a power failure. Basically, an automatic emergency power supply is always necessary if it is not ensured 24 hours a day that the emergency power supply

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can be started manually within a short time. The period in which the emergency power supply must run depends on the technology installed on the biogas plant. The time must be chosen in such a way that no dangerous situations can occur. The most important functions are listed below.

The **gas emergency flare** and the associated gas blower must be integrated into the emergency power supply to ensure the combustion of biogas if the gas storage is full. Since the CHP cannot run without a connection to the power grid, the gas flare is the only way to utilize the gas during power outages. If the gas flare does not work, the gas flows out via the overpressure protection. In this case, explosive areas are created around the overpressure protection.

The **plant control system** must also remain functional in the event of power failures to be able to send fault messages and continue to execute the most important controls. Unlike the other equipment, the PLC must be supplied with power without interruption. For this purpose, additional battery storage units are installed for the PLC for a bridging time of about 10 - 120 min.

The **support air blowers** of double diaphragm gas storage tanks must also be supplied with emergency power. Without running blowers, the supporting air flows out of the space between the gas storage foil and the weather protection foil and the weather protection foil is no longer tightly stretched. As soon as wrinkles form, the membrane becomes very susceptible to gusts of wind. In extreme cases, this can lead to rupture of the gas accumulator.

Especially in plants working with a high DM content or a viscous substrate, the **agitators** must be included in the emergency power supply. Otherwise, the gas bubbles in the substrate cannot escape properly without agitation. This can quickly (approx. 20 min) lead to large increases in volume of the fermenter contents. The fermenter "boils over". This applies to plants that work purely with corn silage or grain silage and a DM content of 10 % or more. In other plants, the problem is less acute. In plants for the exclusive fermentation of liquid manure, the problem does not exist at all.

Which other parts of the plant still need to be supplied with emergency power depends on the plant in question. This can concern, for example, heaters on parts at risk of frost, pumps that protect against overfilling and various other installations.

References

- Anleitung zur Ermittlung des FOS/TAC mittels Titration. http://www.fermenter-doktor.com/FD-web-content/download/B07-02_FermenterDoktor_Titrieranleitung.pdf. (accessed 03.11.2021).
- Arbeitsgemeinschaft Landtechnik und landwirtschaftliches Bauwesen in Bayern e.V. Marktübersicht Zusatz- und Hilfsstoffe in Biogasanlagen. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwjS15jxsIj0AhUeg_0HHcZTAIUQFnoECAgQAQ&url=https%3A%2F%2Fwww.biogas-forum-bayern.de%2Fmedia%2Ffiles%2F0004%2F2zusatz-und-hilfsstoffe-stand-januar-2018.pdf&usg=AOvVaw3YBRG4eUWV5KLTit-R (accessed 08.11.2021).

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Binder Engineering GmbH. COMBIMASS® GA-s hybrid premium. https://binder-group.info/Produktbereiche/combimass-ga-s-hybrid-premium/ (accessed 09.11.2021).
- Bochmann G, Herfellner T, Susanto F, Kreuter F, Pesta G. 2007. Application of enzymes in anaerobic digestion. Water Sci Technol. 56, 29-35.
- Chae KJ, Jang AM, Yim SK, Kim IS. 2008. The effects of digestion temperature and temperature shock on the biogas yields from the mesophilic anaerobic digestion of swine manure. Bioresour Technol. 99, 1-6.
- Drosg B., 2013. Process monitoring in biogas plants. In: Frost P, Baxter D, (eds.). IEA Bioenergy: https://www.nachhaltigwirtschaften.at/resources/iea_pdf/reports/iea_bioenergy_task37_brochure_biogas_process_montoring.pdf (accessed 09.11.2021)
- Fachagentur Nachwachsende Rohstoffe e.V. (FNR). 2010a. Guide to Biogas - From production to use. Gülzow.
- Fachagentur Nachwachsende Rohstoffe e.V. (FNR). 2010b. Gülzower Fachgespräche Band 35 Einsatz von Hilfsmitteln zur Steigerung der Effizienz und Stabilität des Biogasprozesses. ISBN 978-3-942-147-03-3
- Fachagentur Nachwachsende Rohstoffe e. V. (FNR). 2013. Leitfaden Biogas – Von der Gewinnung zur Nutzung.
- Franke L. Möglichkeiten der Gasanalyse in der Biogastechnik. https://docplayer.org/8797104-Moeglichkeiten-der-gasanalyse-in-der-biogastechnik.html (accessed: 10. 11 2021).
- Göller A, Jungstand B, hf sensor GmbH: Mikrowellen-TS-Messung in wässrigen Flüssigkeiten. https://www.ndt.net/article/dgzfp-thz-2017/papers/vortrag4.pdf (accessed 10.11.2021).
- Henkelmann G, Meyer zu Köcker K, Götz J, Beck J. 2010. Biogas – Laborparameter und Prozessüberwachung. Bayerische Landesanstalt für Landwirtschaft – Schriftenreihe, ISSN 1611-4159.
- Kliche R, Lebuhn, M. 2017. Schaum in Biogasanlagen. In: Biogas Forum Bayern Nr. III. 19-2017. https://www.google.com/imgres?imgurl=x-raw-image%3A%2F%2F%2F853a44eba3fce69adc395f4baa591937f9014f9358dba993fc031f2ee6d0693b&imgrefurl=http%3A%2F%2Fwww.biogas-forum-bayern.de%2Fmedia%2Ffiles%2F0003%2Fschaum-in-biogasanlagen.pdf&tbnid=mub8o-tE-OoW2hM&vet=12 (accessed: 11.08.2021).
- Koch K, Post M, Auer M, Lebuhn M. 2017. Einsatzstoffspezifische Besonderheiten in Der Prozessführung. In Biogas Forum Bayern.
- Lili M, Biró G, Sulyok E, Petis M, Borbély J, Tamás J. 2011. Novel approach on the basis of FOS/TAC method. Analele Universităţii din Oradea, Fascicula: Protecţia Mediului, 17, 713-718.
- Lindner J, Zielonka S, Oechsner H, Lemmer A. 2015. Effect of different pH-values on process parameters in two-phase anaerobic digestion of high-solid substrates. Environ Technol. 36, 198-207.
- Lossie U, Pütz P. 2021. Gezielte Steuerung von Biogas anlagen mittels FOS/TAC. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwjJuayu6fvzAhXNiv0HHUXXDxcQFnoECAQQAQ&url=https%3A%2F%2Ffr.hach.com%2Fasset-get.download.jsa%3Fid%3D25593612597&usg=AOvVaw1iA_N3ptfrGQ2ygZu7VYwy (accessed 03.11.2021).
- Meegoda JN, Li B, Patel K, Wang LB. 2018. A review of the processes, parameters, and optimization of anaerobic digestion. Int J Environ Res Pub H, 15, 2224.
- Messtechnik für die Biogasanlage der nächsten Generation: Mit Präzision zur Energiewende. https://www.kemptner.com/files/anwenderreportagen/x-technik/xTechnik_Endress_EVM_AT_1503.pdf (accessed 10.11.2021).
- Nasr N, Elbeshbishy E, Hafez H, Nakhla G, El Naggar MH. 2012. Comparative assessment of single-stage and two-stage anaerobic digestion for the treatment of thin stillage. Bioresour Technol. 111, 122-126.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Otu I. 2020. Biogas as an alternative renewable energy source to reduce the harmful effects of traditional biomass on the environment in asamanskese. 10.13140/RG.2.2.30007.96164.
- Postel J, Jung U, Fischer E, Scholwin F. 2009. Stand der Technik beim Bau und Betrieb von Biogasanlagen. Umweltbundesamt. ISSN 1862-4804.
- Reinhold G. Masse- und Trockensubstanzbilanz in landwirtschaftlichen Biogasanlagen. http://wiki.istanbullisesi.net/images/3/35/Biogasgewicht3.pdf (accessed 04.11.2021).
- Schlegel M, Kanswohl N, Rössel D, Sakalauskas A. 2008. Essential technical parameters for effective biogas production. Agron Res. 6, 341-348.
- Singlitico A, Dussan K, O'Shea R, Wall D, Goggins J, Murphy J, Monaghan RF. 2017. Techno-economic optimisation of combined anaerobic digestion and gasification of food waste as part of an integrated waste management and energy system. In Proceedings of the 25th European Biomass Conference and Exhibition, Stockholm, Sweden (pp. 12-15).
- Song YC, Kwon SJ, Woo JH. 2004. Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic-and thermophilic digestion of sewage sludge. Water Research. 38, 1653-1662.
- Weiland P. 2010. Biogas production. Current state and perspectives. Appl Microbiol Biotechnol. 85, 849–860.

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3. Economic and ecological aspects

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Abstract: From an economic perspective, anaerobic digestion (AD) is a valuable process that generates a renewable energy source with the production of biogas as the energy carrier. It also adds value to organic waste as feedstock of the process. Biogas is a mixture of gases, mostly carbon dioxide (CO_2) and methane (CH_4), which is the most valuable compound since its combustion releases energy that can be used to produce electricity and heat, or as a replacement for natural gas. In addition, the digestate, which is a co-product of the process, can be used as a fertilizer in the agronomic industry. In this way, AD is a powerful tool to promote circular economy systems, producing a sustainable energy source and recycling organic waste that returns to the economic circuit in the form of fuels or fertilizers.

From an environmental perspective, biogas production through AD can help to mitigate four important environmental concerns of 21st century: climate change, overuse of synthetic fertilizers, methane emissions and waste management. AD produces a renewable energy source (biogas) with a net balance of zero-carbon release into the atmosphere. This positive effect on the reduction of greenhouse gases (GHG) emissions is even enhanced considering that if waste is not treated, it is also a source of GHG, mostly methane. Also, it is a sustainable management practice for the enormous amounts of waste that are constantly generated due to human activities, while most of the current practices are not sustainable at all. Finally, the use of the digestate as organic fertilizer allows the recycling of nutrients, especially nitrogen, with further positive impacts on the environment, and helps to promote agroecology and bioeconomy.

3.1 The economic biogas framework in Europe

The biogas and biomethane production have been growing steadily worldwide as well as in Europe, and incentives for the use of this renewable energy have been gaining ground within public policies. According to the Renewable Energy Directive, by 2030, 32% of all energy used in the EU must come from renewable sources, including biomass, bioliquids and biogas. In addition, European countries must establish a national action plan with the objective of defining the share of energy from renewable sources as well as establishing procedures for the reform of planning and pricing scheme and access to electricity networks, promoting energy from renewable sources. (Biogas3, 2016).

More generally, incentives in the biogas sector seek to implement a sustainable bioeconomy that represents the renewable segment of the circular economy, capable of transforming organic waste into valuable resources and creating innovations and incentives to help reduce of food waste and improve the organic waste treatment (Communication, 2018).

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As a product, biogas constitutes an economy generator center in Europe, especially because it is considering a consumer of a significant supply chain, presenting concrete economic results in the form of energies, carbon credits and energy efficiency, which constitute economic revenues in the biogas economy. The demands of this economy are prerequisites for biogas generation to be implemented, such as projects, environmental licensing, regulation, technical training, and others. In addition, the biogas sector moves a supply chain which is necessary for a biogas plant to be installed and operated, such as engines, generators, controls, biodigesters, filters, pipes and several other parts, components, and processes of industrial origin, which drive commerce and specialized services. The economic results that come from the biogas economy are consider direct, such as electric, thermal, automotive energy, and digestate applied for self-consumption and for the sale of surplus, and indirect, such as obtaining carbon credits for reducing gas emissions from greenhouse effect, and the environmental protection through the polluting organic loads reduction and its energy efficiency (Fagerström, et al., 2018).

However, according to current trends, biogas production still depends on subsidies to attract investors and establish substantial scale. In the EU, there is still no specific policy on biogas, biogas solutions are addressed in various policy documents and directives related to renewable energy and bioenergy such as the Waste Directive (Directive 2018/851; Directive 2008/98/EC), EU Bioeconomy Strategy (Communication, 2018), Renewable Energy Directive (Directive 2018/2001; Directive 2009/28/EC) and Landfill Directive (Directive 1999/31/EC), but there were no major initiatives aimed directly at the development of biogas. Currently, some new strategies are emerging in which biogas solutions play a central role. As an example, the EU Methane Strategy (Communication, 2020) emphasizes the importance of biogas production to reduce methane emissions from the agriculture, waste and energy sectors, and the Energy System Integration Strategy (Communication, 2020) pronounces the mobilization of residual resources for energy production and replacement of fossil gas with renewable gases. However, despite the various incentive schemes to stimulate the economic development of the biogas sector (certification systems, feed-in tariffs, and investment support), the implementation process depends on several factors, including national market conditions (energy prices), tax regimes, economic policy, technical, institutional, sociocultural, and environmental factors (Nevzorova and Kutcherov, 2019).

Thus, the fact that biogas solutions can provide many benefits in different sectors of the economy and contribute to the sustainable solution can be considered an advantage, but the many functions and externalities also make biogas solutions complex to evaluate (Gustafsson and Anderberg, 2022).

3.2 Exemplary biogas frameworks of selected countries

3.2.1 Finland

Finland is a prominent country in the use of renewable energies (Lyytimäki et al., 2018), and has a growing development in the biogas sector. However, due to the high costs regarding

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the conversion of biogas into biomethane, the country has a much lower biomethane production compared to biogas. Thus, in order to support the biogas and biomethane production, Finland has developed new tariff and tax systems which both biogas and biomethane are exempt from excise duties in all end-use applications (EBA 2021; (Winquist et al., 2021). Nevertheless, the taxation of biomethane as a vehicular fuel is now under discussion.

Currently, the country has a biogas and biomethane production potential of 10 TWh, with a theoretical potential of 25 TWh (Biokaasu2030) and with the purpose of implement improvements and increase production, the Finnish biogas sector set a target of reaching an annual production of 4 TWh by 2030, which was confirmed by the government in September 2021 (EBA, 2021).

Due to the different biogas incentive strategies used by the government, the number of biogas plants increased considerably, reaching more than 38 plants between 2011 and 2021 (Biokaasu2030). Presently, Finland has a total of 113 plants that use biomass from different sources such as landfills (34 plants), urban solid waste (26 plants), agriculture (25 plants), sewage treatment sludge (19 plants), and industrial biogas (9 plants) (**Figure 51**). In addition, among them, the urban organic waste and sewage sludge plants are responsible for presenting a greater amount of biogas production (EBA, 2021). However, despite the biomass from the agricultural sector presenting the highest generation potential, only a small part is treated in biogas plants (Huttunen et al., 2018).

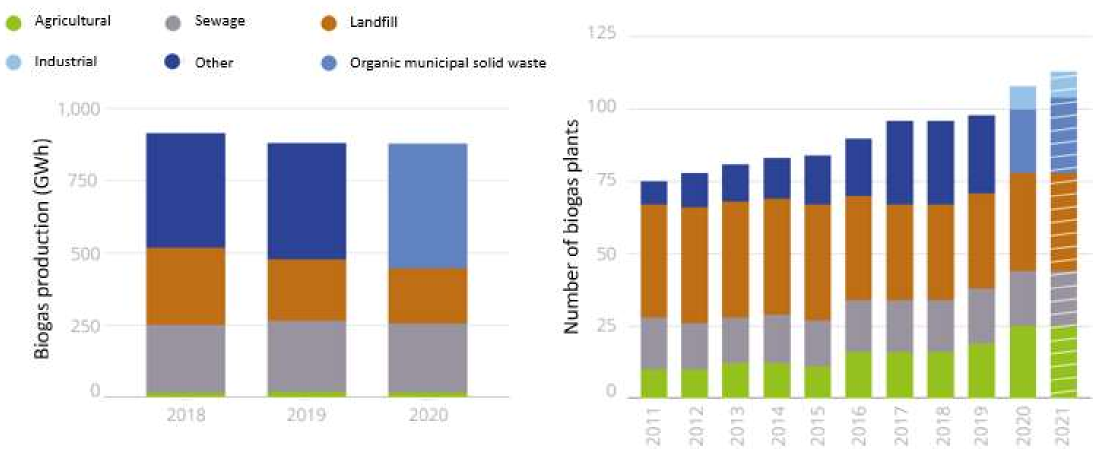

Figure 51: Development of biogas production (GWh) (left); and development of the number of biogas plants (right). (EBA, 2021)

In 2020, Finland has reached a production of 878 GWh of biogas, and it is estimated that approximately 60% of the biogas produced is used as thermal energy for heating or sold directly as raw biogas, while the other part is consumed in cogeneration. (EBA, 2021).

As well as the biogas sector, despite slower growth, the biomethane sector presents a promising development due to the support policies that have been implemented in recent years

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by the Finnish government, and mainly due to the Finland's national biogas action plan published in 2020, which describes all the measures and actions that will support the sector until 2024 (Biokaasu2030). This can be observed between 2011 and 2021, where the number of biomethane plants increased in the country from 1 to 22, reaching a production of 109 GWh in 2020, with most biomethane plants using solid urban waste as a source of biomass. (EBA, 2021).

However, although the number increase of biomethane plants, currently, only 40% of plants are connected to the biogas grid. This is because only the southern part of the country has a gas network enabling direct injection into it.

In general, due to the great political incentive to promote biogas for the use of transport fuel, the production of biomethane is growing. Thus, from 2022 it will be part of the national biofuel delivery obligation, giving a stable perspective to increase the production and use of biomethane by 2030 (EBA, 2021; (Biokaasu2030).

Influence of the biogas sector on GHG emissions

According to the National Inventory Report, the total greenhouse gas emissions in 2020 was 47.8 million tonnes of carbon dioxide equivalents (Mt CO_2 eq.). However, compared to the base year (1990) and 2019, emissions decreased by 33% (23.4 Mt) and 9% (5 Mt) respectively.

Table 21: Finnish greenhouse gas emissions and removals (Mt CO_2 equivalent) (NIR, 2020)

Sector	**Base year**	**1990**	**1995**	**2000**	**2005**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**
Mt CO_2 eq.																
Energy	53.4	53.4	55.3	53.7	53.7	60.2	52.8	47.5	48.1	44.3	40.6	43.3	40.9	42.1	38.9	34.3
Industrial processes and product use	5.3	5.3	4.9	5.2	5.6	4.8	4.7	4.6	4.4	4.2	4.4	4.7	4.6	4.6	4.4	4.1
F gases	0.1	0.1	0.2	0.7	1.2	1.4	1.4	1.4	1.4	1.3	1.3	1.2	.1.	1.1	1.0	1.0
Agriculture	7.5	7.5	6.7	6.6	6.5	6.7	6.5	6.4	6.5	6.6	6.6	6.7	6.6	6.5	6.6	6.6
Waste	4.7	4.7	4.6	3.8	2.8	2.6	2.5	2.4	2.3	2.2	2.1	2.0	1.9	1.8	1.8	1.7
Indirect CO_2 - emissions	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TOTAL	**71.2**	**71.2**	**71.8**	**70.2**	**69.9**	**75.7**	**67.9**	**62.4**	**62.8**	**58.6**	**55.0**	**57.9**	**55.1**	**56.2**	**52.8**	**47.8**

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Regarding the different sectors responsible for gas emissions in Finland, it is clear that the energy sector is considered the most significant source among them (**Table 21**) with a variation in CO_2 emissions according to the economic trend, the supply structure of energy, and weather conditions ((Finland. 2020 National Inventory Report (NIR) | UNFCCC). However, over the years, due to some changes related to the level of electricity imported annually, the consumption of energy based on fossil fuels and the growth in the use of renewable energy, greenhouse gas emissions have decreased considerably (IEA, 2021)..

In 2020, emissions from the energy sector fell by 12% to 34.3 Mt CO_2 eq. from the previous year. Emissions were 36% below the 1990 level and 51% below the 2003 level (Finland. 2020 National Inventory Report (NIR) | UNFCCC)

This way, the renewable energy consumption has been growing steadily in the country as well as the development of the biogas sector, representing 44.6% of the total consumption of final energy. In 1990, the share of renewable energy was only 18%, showing that the increase in the use of renewable energy was the main reason for the decrease in gas emissions, despite the growth in total energy consumption (NIR, 2021).

3.2.2 Spain

Spain started to support renewable energy in 1997, through the "General Electricity Law 54/1997"; however, the country still has a very strong dependence on energy imports, dominated by oil and Natural Gas (IEA, 2019). With the implementation of a national policy mechanism that provide payments and long-term contracts to renewable electricity producers called Feed-in Tariff (FIT), biogas production has increased considerably in the country (EBA, 2021; (Del Rio, 2008)). Unfortunately, according to the EBA 2021 report, the records referring to the number increase of biogas plants do not reflect reality, as many of the plants that already existed in the past were not registered due to lack of data. However, the growth in the number of biogas plants in Spain is believed to have largely occurred between 2000 and 2004, mainly with the use of sewage and sanitary landfills for biogas production. In addition, after the enactment of Royal Decree Law 1/2012, which eliminated all incentives aimed at generating electricity from renewable sources, the commissioning of new plants decreased

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dramatically (EBA, 2021; (Flórez and Isabel, 2017). **Figure 52** shows biogas production and the reported number of biogas plants in Spain during the last decade.

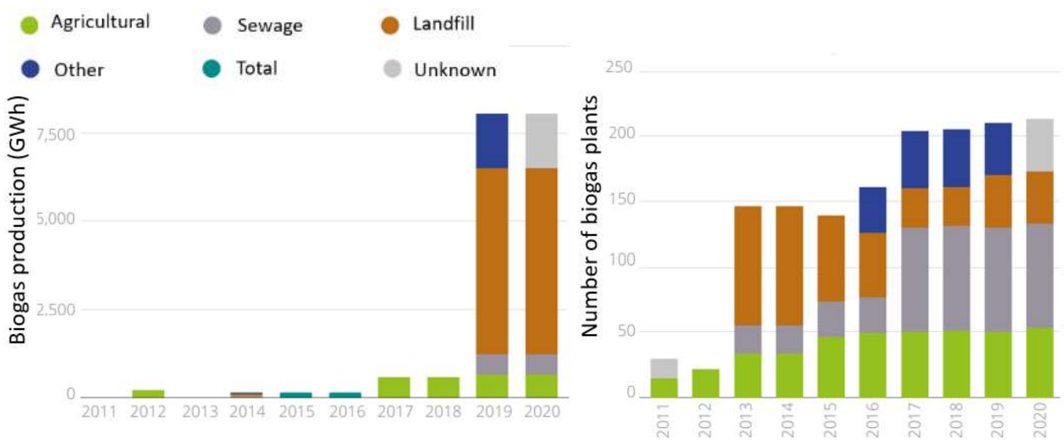

Figure 52: Development of biogas production (GWh) (left); and development of the number of biogas plants (right) in Spain (EBA, 2021)

Furthermore, the Royal Decree Law 413/2014 establishment, which regulates the calculation of feed-in tariffs (FIT) for the electricity production from biogas together with the hydrocarbons tax applied to biogas (0.65 €/ GJ), helped to further reduce activities in the biogas sector (Romero-Rubio and de Andrés Díaz, 2015; Ministerio de Industria, Energía y Turismo, 2014). Currently, Spain has no incentive for new projects related to biogas production. However, there are still projects regarding to the biogas generation, which are not encouraged by the potential for selling biogas, but rather they are driven mainly by the needs of waste treatment and/or by the prospect of providing energy for private consumption (EBA, 2021).
Today, Spain has 210 active biogas plants, the majority coming from sewage treatment plants, followed by agriculture, landfill and others. With this number of biogas plants, the country has a total biogas production capacity of 836 MW, corresponding to 318 MW of installed electrical capacity (EBA, 2021).
On the other hand, Spain also has three biomethane plants placed, with two of them came into operation in 2021 (EBA, 2021). In addition, due to the incentive associated with private initiatives (R&D) and partly funded by the EU (LIFE, H2O2 and CEF2), several pilot-scale projects are growing. Currently, the Spanish Ministry of Ecological Transition and Demographic Challenge (MITTERD) has approved the first Law on Climate Change and Energy Transition of Spain in the first half of 2021, where it commits the country to reduce emissions by 23% until 2030, comparing to the levels of 1990 (BOE, 2021). With this achievement, the government has the possibility to approve mechanisms to support renewable gas and its injection into the gas grid. The law also approves the guarantees registration of origin (GOs) for renewable gases injected into the natural gas network (BOE, 2021).

This way, with more support and encouragement of the government, Spain can exceed 100 TWh per year if every type of raw material available is exploited, increasing biomethane production (EBA, 2021).

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<u>Influence of the biogas sector on GHG emissions</u>

According to the NIR report (2021), total greenhouse gas emissions (GHG) in Spain estimated for 2019 were 314,528.5 kilotons of CO_2 equivalent (CO_2-eq). This represents a reduction of -5.6% in relation to the estimated emissions for the year 2018. Moreover, it constitutes +8.5% in relation to the base year 1990 and -28.9% in relation to the year 2005. In Spain, the gases that showed the highest emission rates in 2019 were CO_2 (80%) and CH_4 (12.2%) (**Tables 22 and 23**). However, comparing the different sectors, all of them suffered a drop in GHG emissions, the main being related to electricity generation (-27.7 %), to the commercial and residential sector (-8,6 %), and to the industrial sector (-1.2 %).

Table 22: *CO_2 emissions: absolute values, temporal variation and ratios (NIR, 2021).*

	1990	2005	2010	2015	2018	2019
CO_2 (kt CO_2-eq)	231.194	369.681	283.873	271.694	269.713	251.498
Variation % vs. 1990	100%	159.9%	122.8%	117.5%	116.7%	108.8%
CO_2 / INV (CO_2-eq)	79.7%	83.6%	79.3%	80.6%	80.9%	80.0%

Table 23: CH_4 emissions: absolute values, temporal variation and ratios (NIR, 2021).

	1990	2005	2010	2015	2018	2019
CH_4 (kt CO2-eq)	36.647	40.924	39.462	38.177	38.566	38.493
Variation % vs. 1990	100%	111.7%	107.7%	104.2%	105.2%	105.0%
CH_4 / INV (CO_2-eq)	12.6%	9.3%	11.0%	11.3%	11.6%	12.2%

In general, the evolution of CO_2 and CH_4 emissions in Spain over time responds to a four-phase pattern fundamentally linked to variations in economic growth, population, or energy consumption in Spain since 1990 (IEA, 2021; NIR, 2021). However, the decrease in emissions was mainly marked by the reduction in emissions from electricity production (-27.7 %), due to the greater production of renewable energies, such as wind, photovoltaic, solar thermal and biomass, which increased +9.4%, +19, 0% and +16.8% respectively, and the decrease in the use of coal in electricity production (-66.0%) (IEA, 2021; NIR, 2021). The energy sector accumulates a total GHG reduction of -6.6% (NIR, 2021). This represents a significant reduction in GHG emissions compared to the increase in the renewable energy use, showing that with incentives through public policies, and the appropriate treatment of organic solid waste significantly influences the biogas sector growth and the preservation of the planet.

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3.2.3 Netherlands

In the biogas market, the Netherlands is one of the countries that has stood out significantly throughout the world due to the implementation and upgrading of biogas plants to large-scale biomethane plants, presenting strategies well established by the government in partnership with the private sector (IEA, 2021; (Winquist et al., 2021).

According to Kwant, 2003, the Netherlands had its first support for encouraging the renewable energies use in 1995 with the Green Funds certification. However, the biogas sector only showed significant growth in 2006, 3 years after the introduction of the "Environmental Quality of Electricity Production" Feed-in-Premium (FIP) which helped to support the implementation of new plants (EBA, 2021). In addition, with the goal of stimulating the production and cogeneration of renewable energy, the SDE (Stimuling Duurzame Energie) was created. This was later updated to the SDE+ and SDE++ that are currently being used to encourage sustainable growth and the circular economy through subsidies from the government (Netherlands, 2022; IEA, 2021). As a result, there was an increase in the number of biogas plants (260 in 2020) and biomethane plants (60 in 2020), showing the importance of implementing efficient laws driven by the government (EBA, 2021). Thus, the Netherlands produced 2,439 GWh of biogas in 2020, of which approximately 927 GWh of electricity was generated (**Fig 53**) (EBA, 2021).

Figure 53: Development of biogas production (GWh) (left); and development of the number of biogas plants (right) (EBA, 2021).

Due to government incentives for biogas production, the biomethane market has also grown significantly, making the Dutch market in this sector one of the pioneers in Europe (Winquist et al., 2021). The Netherlands currently has its own national renewable gas registry operated by Vertogas, the green subsidiary of Nerderlandse Gasunie, since 2009, and registration and certification via Vertogas is mandatory for renewable gas producers in the country (Vertogas). Because of this incentive, the number of biomethane plants grew from 21 in 2016 to 60 plants in 2020, with agricultural substrates as the most used feedstock, followed by industrial

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waste. Furthermore, in 2020 the Netherlands reached a production if 2,166 GWh of biomethane (EBA, 2021). Today, it is known that out of the 60 biomethane plants in the Netherlands, 53 are connected to the gas grid, 4 plants produce Bio-CNG on site and have no connection to the grid, one produces Bio-LNG, and the others do not have record (IEA, 2021; EBA, 2021).

However, it was pointed out in 2020 the production of 402 GWh of biomethane which were used in transport representing practically 1/5 of the biomethane produced in the same year. Due to the large investment in the production of vehicular biofuel, 7 new Bio-LNG plants are planned for the period 2021-2024 with a total capacity of 1.5 TWh per year (IEA, 2021; EBA, 2021).

Influence of the biogas sector on GHG emissions: Another important factor related to the growth influence in the dutch biogas sector is the significant reduction in greenhouse gases (GHG). According to NRI (2021), the energy sector is responsible for 83% of total GHG emissions in the Netherlands, being considered the most important source of GHG emissions. However, in 2019, total GHG emissions in the Netherlands were estimated at 180.7 Tg CO_2-eq. This is 18% lower than the 220.5 Tg CO_2-eq. reported for the base year (1990) (**Figure 54**).

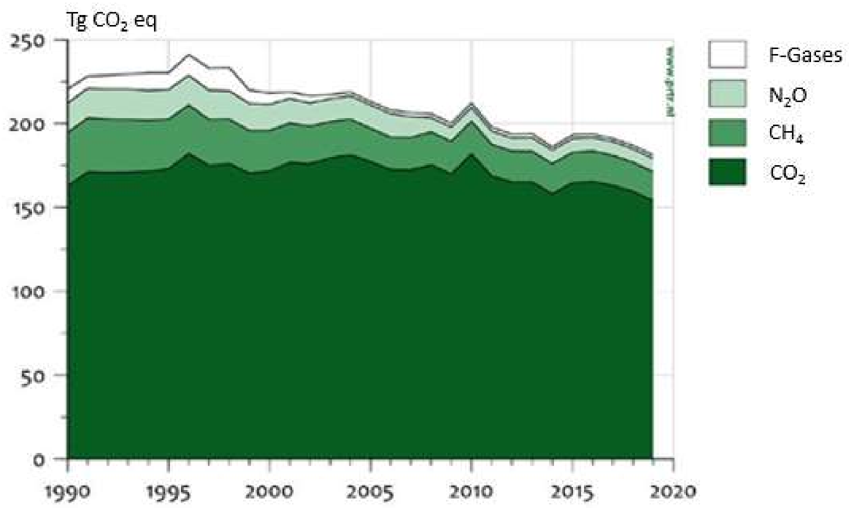

Figure 54: Greenhouse gases: emission levels and trend, 1990-2019 (NIR, 2021)

In the period 1990-2019, emissions mainly of carbon dioxide (CO2) and methane (CH4) decreased by 5.6%, and 45.9%, as well as nitrous oxide (N2O) and fluorinated gases 54.9% and 75.9%, respectively.

However, despite the use of fossil fuels has been decreasing and the amount of energy from renewable sources has been increasing, natural gas (44%) and oil (36%) are still the most important energy sources in the Netherlands (IEA, 2021). **Figure 55** shows the mix of renewable energy sources in the Netherlands. Renewable energies accounted for 181 PJs in 2019 (8.7% of total energy use in the Netherlands).

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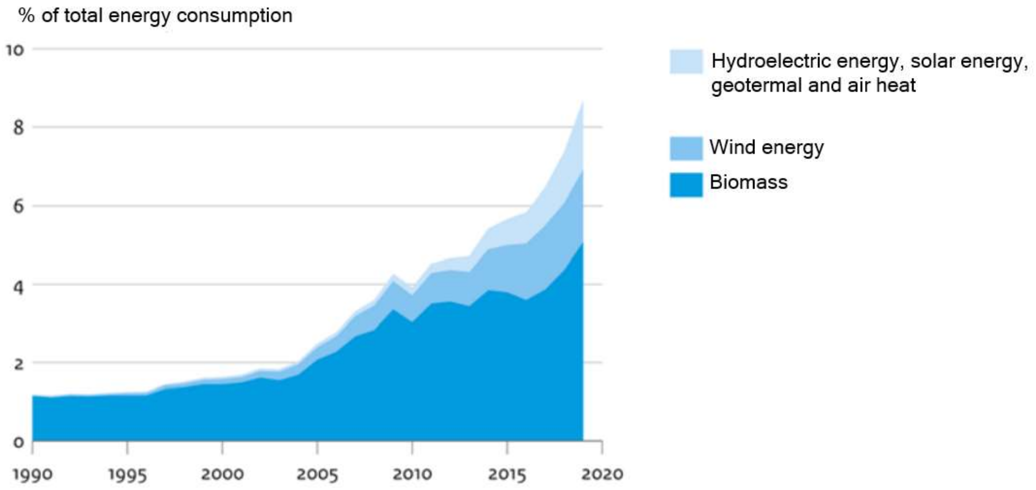

Figure 55: Development of renewable energy as a percentage of total energy demand in the Netherlands, 1990–2019 (CLO, 2020).

3.2.4 Belgium

Before 2001, the biogas sector in Belgium was stagnant and consisted mainly of plants operated in landfills or sewage treatment located in the region of Brussels, Wallonia and Flanders (EBA, 2021). However, after the introduction of the Green Certificate Scheme project by royal decree, the biogas sector grew considerably (Marchal et al., 2007). The Green Certificate determined the electricity share from renewable sources delivered to users connected to the distribution network (from 2% in 2002 to 6% in 2006), and a fine of 75 euros per certificate for non-compliance with the quota (corresponding to 1 MWh) (EU Comission, 2018). Between 2015 and 2018, due to technical reasons, several small biogas plants had to be closed in the Flanders region, but with the effort of the sector to solve this problem, the growth rate of plants grew again in 2019 (**Figure 56**) (EBA, 2021). Currently, Flanders and Wallonia are the regions that most biogas produce in Belgium, with 134 and 55 plants in operation. In 2020, the country reached a production of 2700 GWh resulting in the generation of 1075 GWh of electricity (EBA, 2021).

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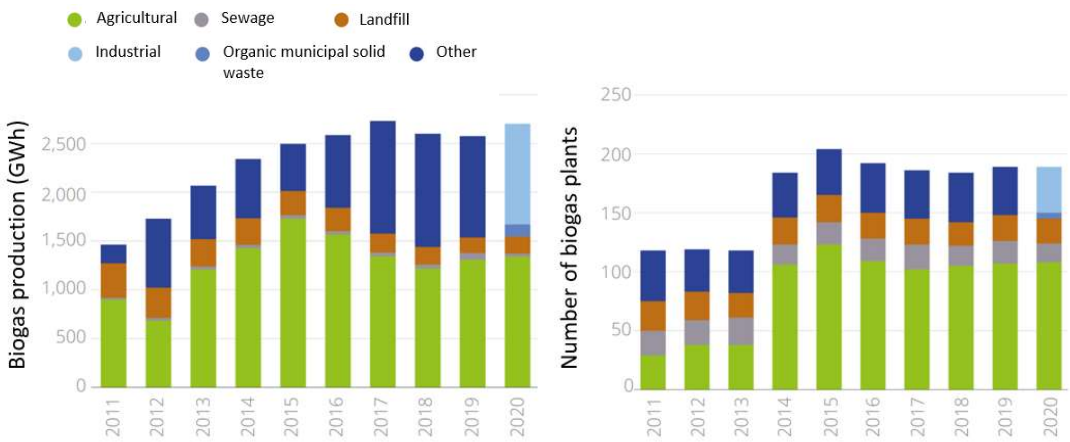

Figure 56: Development of biogas production (GWh) (left); and development of the number of biogas plants (right) (EBA, 2021).

In addition to biogas plants, interest in biomethane production increased in the country after the installation of the first biomethane plant in the Flanders region, which helped to encourage policy changes across Belgium (IEA, 2021). In Wallonia, the system to support the use of green energy has changed to include the biomethane production, for which the company can receive a government subsidy by reducing the use of fossil fuels. Furthermore, Wallonia has taken on the "Plan wallon Energie Climat" (Wallonia regional climate plan) to help achieve its 2030 targets for biogas development. A voluntary registration system for renewable gases was also created by the association of gas distributors in Belgium (Gas.be) to help with the incentive in the sector (Biogaz). In September 2021, Belgium had the registration of the first Bio-CNG produced in Wallonia, however a second plant is being built in Flanders (EBA, 2021).

Currently, 6 biomethane plants are in operation in Belgium, where 2 are in the Flanders, and 4 in the Wallonia region. Of these 6 plants, 3 are operating with agricultural substrates, one producing biomethane from sewage sludge, one using organic municipal solid waste and an industrial biomethane plant. It is estimated that today Belgium reaches about 17% of its biogas and biomethane production potential (EBA 2021).

Influence of the biogas sector on GHG emissions: As well as the other countries, the biggest contribution to GHG emissions in Belgium comes from the energy sector. In 2019, the sector contributed 74% to total GHG emissions. Mainly, CO_2, CH_4 and N_2O emissions come from the energy sector. However, since 1990, emissions from this sector have declined by around 17% (NIR, 2021).

Another largest source of greenhouse gases is agriculture with 8%, where emissions from this sector arise mainly from CH_4 and N_2O. However, since 1990, emissions have fallen by 19%. In 2019, the waste sector contributed around 1.2% where emissions arise from CO_2, CH_4 and N_2O and originate from waste incineration, solid waste disposal on land and wastewater treatment. Emissions from this sector have steadily declined and are 69% below 1990 level since 2019. According to the National Inventory Report from Belgium, the total net emissions have decreased by 18.8% since 1990.

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This way, it is evident that the emissions decrease in the energy, agricultural and waste sectors in Belgium is due in part to technological improvements, the shift from solid fuels (coal) to gaseous fuels (natural gas) and the use of renewable energies, such as the biomass for biogas and biomethane production, as well as the proper treatment of urban solid waste using anaerobic digestion.

3.2.6 Greece

Greece began to explore energy using biogas in the early 1980s. The main raw material was animal waste and food-processing industries waste. However, due to a lack of adequate legislation, financial incentives and public awareness, the projects ended up being forgotten (Markou et al., 2017).

From 2000 to 2010 the country started to produce biogas again, which was dominated by plants that used sewage and sanitary landfills as a source of substrate. Between 2011 and 2020 there was an increase in biogas production from 543 GWh to 718 GWh, most of which came from Athens and Thessaloniki (EBA, 2021; IEA, 2021). With the introduction of Feed-in Tariffs (FiTs) in its Renewable Energy Act in 2010, fees for landfill-based biogas plants were up to 120 euros/MWh, while for agriculture-based biogas plants they were up to 220 euros/MWh, which resulted in an increase in agricultural biogas production (EBA, 2021; (Markou et al., 2017)). Later, with the increase of the maximum tariff rates (FiT) to 129 euros/MWh (landfill biogas) and 225 euros/MWh (agricultural-based biogas), Greece further increased the number of plants, mainly biogas plants using agricultural substrate, reaching a production of 392 GWh in 2020 related to this type of plant (Markou et al., 2017). Due to these changes, that year the country increased by 42 plants with a total production of 1,126 GWh (**Figure 57**) of biogas, from which 428 GWh of electricity were generated (EBA, 2021).

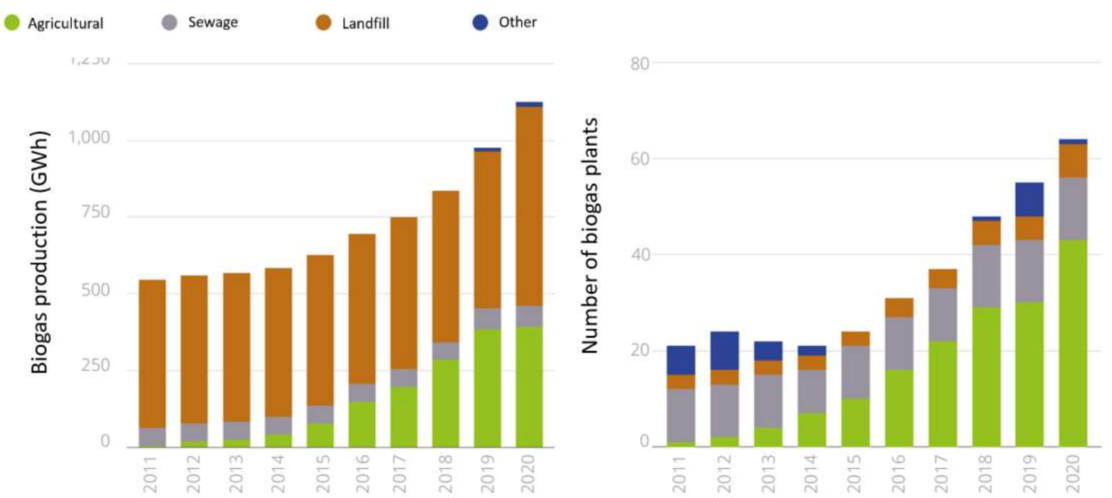

Figure 57: Development of biogas production (GWh) (left); and development of the number of biogas plants (right) (EBA, 2021)

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However, biomethane production has lagged due to lack of incentive through public policies. It is estimated that there will be no significant growth within the next 2 years in this area. In order to have and improvement in this sector, the government must encourage through tariffs (FIT), for example, with generous prices for production as well as facilitating the injection of biomethane into the gas network (Rutz 2021).

<u>Influence of the biogas sector on GHG emissions:</u> According to the National Inventory Report from Greece, in 2019 GHG emissions totaled 85.63 Mt CO_2-eq, showing a reduction of 17.10% compared to 1990 levels. Carbon dioxide emissions represented 76.77% of total GHG emissions in 2019 and decreased by approximately 21.20% compared to 1990. Methane emissions accounted for 11.70% of total GHG emissions in 2019 and decreased by 9.29% compared to 1990.

The energy sector represented 71.50% of total GHG emissions and decreased approximately 20.51% in 2019 compared to 1990 levels. This is due to the improvement in living standards, due to economic growth, the significant growth of the services sector and the introduction of natural gas into the Greek energy system for the period 1990 to 2007.

Emissions from the waste sector have decreased by around 0.52% since 1990. Improved living standards have resulted in an increase in waste generation and therefore in emissions since 1990. However, the increase in recycling along with exploration of the biogas produced limits the increase in methane emissions (NIR, 2021). In this way, it is possible to realize the importance of the biogas sector development for the reduction of negative environmental impacts and the economic improvement of the country.

3.2.7 Germany

Germany is considered the leading country both in Europe and in the world regarding production of biogas and biomethane (Poeschl et al., 2010). With around 11000 biogas plants and 242 biomethane plants currently installed, it is responsible for more than half of the total primary energy produced from biogas. This fact is largely explained by the large state incentive about plantations for the energy production from biomass (WBA, 2021 (Zlokower, 2019; EBA, 2021).

With several laws encouraging the biogas and biomethane production, Germany was one of the first countries to implement a subsidy for the renewable electricity generation, which was later called the Renewable Energy Sources Act (EEG or Eneuerbare-Energien-Gesetz) and came into force in 2000 (Büsgen and Dürrschmidt, 2009). This document regulates the connection of energy production units from renewable sources to the distribution network, as well as the purchase, transmission and payment of energy by the network operator. These differ according to the type of renewable energy, conversion technology and production unit capacity. After the implementation of this law, biogas production in Germany tripled (EBA, 2021). However, over time after the updates of this law, remuneration rates became less generous causing the growth rate of the number of biogas plants to decrease (Matschoss et al., 2019). Furthermore, with the abandonment of feed-in tariffs (FIT) that helped to boost

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the biogas market, Germany implemented a tendering system (EEG-2017) that focuses electricity production on a market-driven rationale (Thomas, 2019). With this change, biogas production has decreased, dropping to 71 TWh in 2020 compared to a production of 81 TWh in 2017 (**Figure 58**) (EBA, 2021).

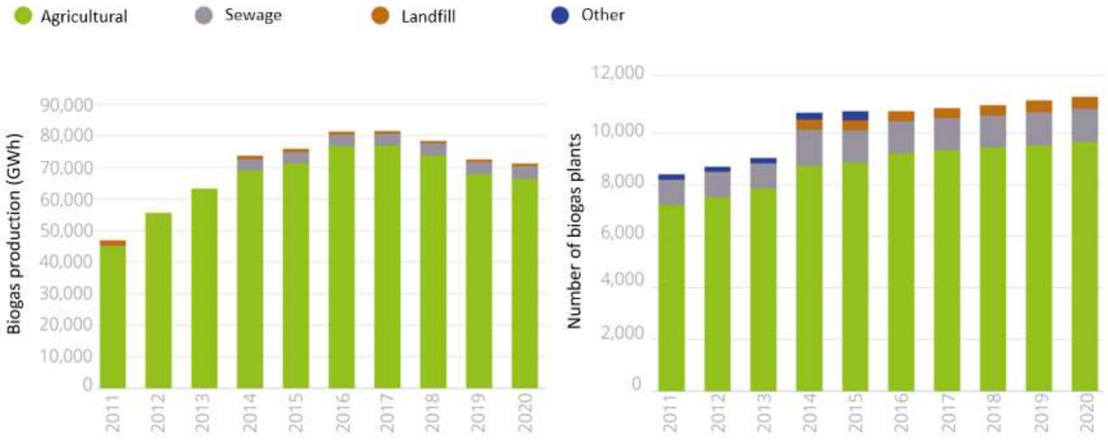

Figure 58: Development of biogas production (GWh) (left); and development of the number of biogas plants (right). (EBA, 2021).

However, despite the decrease in biogas production, installed electrical capacity in the German biogas industry continues to grow yearly due to the flexibilization of Germany's biogas plants. Currently, the latest EEG updates have prioritized smaller biogas plants that use higher proportions of manure, biowaste and food waste, focusing more on ecological sustainability (Thomas, 2019)

Germany is also one of the largest producers of biomethane in the world with 242 registered plants and more than 11 TWh of biomethane production in 2020. After changes in German legislation, the increase in the number of plants has slowed down in recent years, making France overtake Germany as the fastest growing country in the biomethane sector with 306 plants registered in 2021 (EBA, 2021).

Among the biomethane plants registered in Germany, 197 use agricultural substrate, which is the main pillar of the German biogas sector, followed by the organic municipal solid waste (EBA, 2021). The main end-use application of biomethane in Germany is the electricity generation, followed by use for vehicle fuel (Torrijos, 2016). In addition, most biomethane plants in Germany in 2020 were connected to the gas grid, of which 2 were connected to the distribution grid and 104 to the transmission grid (EBA, 2021).

It is estimated that around 1000 GWh of biomethane produced in 2020 was used as transport fuel, representing around 9% of total production. Due to the implementation of the Federal Pollution Control Act, which requires fuel companies to reduce their carbon footprint, the incentive to produce vehicular fuel from biomethane has increased. Germany, together with Italy and the Netherlands, is expected to become a leader in the European production of Bio-LNG (EBA, 2021).

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The largest plant under development in Europe is in Germany, which will have a production capacity of 1500 GWh/year and is expected to start operating in 2022. In addition, the number of Bio-CNG and Bio-LNG filling stations in Germany also is increasing rapidly. Currently, of the 810 CNG stations in operation, 533 supply Bio-CNG (EBA, 2021). In addition, to encourage the production of vehicular fuel through renewable sources, in October 2021, the country obliged producers and suppliers of fossil fuels and non-sustainable biomass to purchase a CO2 Emission Certificate for each ton of CO2 that they release (New CO_2 emissions tax in Germany). Thus, making Bio-LNG a much more attractive, ecological and economical option.

Influence of the biogas sector on GHG emissions: Among other factors, considering the policies to encourage the renewable energy use and the biogas sector development in Germany, it is possible to notice a significant reduction in the levels of GHG, mainly CO_2, which represents the largest share of greenhouse gas emissions (NIR, 2021). The CO_2 are being mostly released through the fossil fuels combustion, and CH_4, released predominantly by livestock, fuel distribution and landfills. **Figure 59** shows a reduction of approximately 35.1% in total GHG emitted compared to the base year (1990), which represents an approximate reduction of 32.4% in CO_2 and 58.2% in CH_4 in 2019 (NIR, 2021).

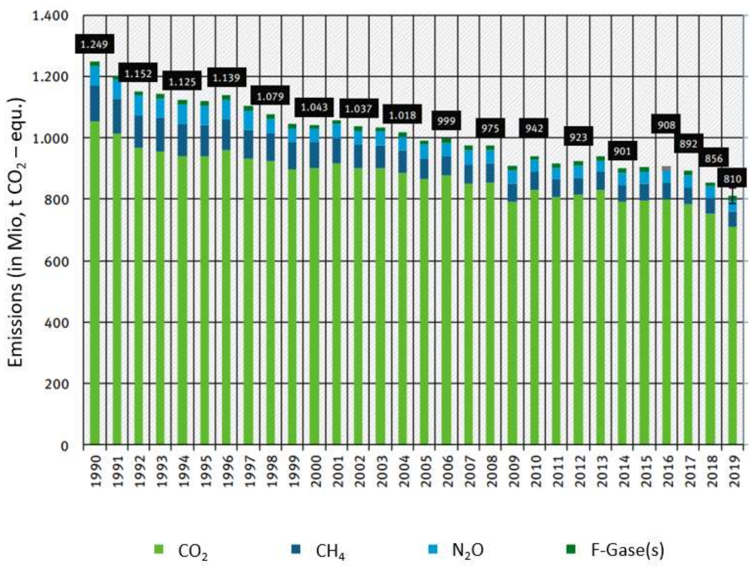

Figure 59: Development of greenhouse gases in Germany since 1990, by greenhouse gases. (NIR, 2021)

The observed reduction is largely due to structural changes in the energy sector, as well as climate and price effects. Furthermore, in recent years, coal has been increasingly supplanted by natural gas, which has lower specific CO_2 emissions in electricity generation, and the share of renewable energies in electricity generation has largely increased considerably, mainly the energy produced through the biogas sector, which has gradually influenced the reduction of CO_2 and CH_4, respectively (NIR, 2021).

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3.3 Ecological and economic impacts of the biogas industry

In this section, we ask which are the main environmental issues that can be mitigated with anaerobic digestion (AD) and biogas production. We state the current situation of the identified issues, together with a theoretical description that helps to understand the basic aspects of each one. Also, we ask how AD and biogas production can take profit of these issues as an opportunity to increase its adoption as a technology for renewable energy production, for sustainable waste management, and for organic fertilizer production. We continue with a description of the concepts of circular economy and bioeconomy, again with AD of organic matter as a pivotal approach that can foster these production and economical systems. Finally, we explain the life cycle assessments (LCA) and how can they be implemented for biogas projects.

3.3.1 Current Status

An ecosystem can be defined as a biological system constituted by live organisms (biotic factors) and the physical medium that they share and where they live in (abiotic factors). From the point of view of a single species, the environment is defined as the biological, physical and chemical components of the ecosystem with which this specie interacts. The lack of awareness in the modern era about the human species as a biological factor of the ecosystems, has led to the underestimation of the consequences of human actions over the rest of the biotic and abiotic factors, this is, over the environment. These environmental consequences are also translated into economic consequences.

In the Resolution of October 15th 2019, the General Assembly of the United Nations recognizes that in the actual world *"(...) inequalities in wealth, incomes and opportunities are increasing in and between countries. Biodiversity loss, environmental degradation, discharge of plastic litter into the oceans, climate change and increasing disaster risk continue at rates that bring potentially disastrous consequences for humanity*". In the same resolution, the United Nations reaffirm the commitment to the 2030 Agenda. In this Agenda, the 17 Sustainable Development Goals, which guide policies to achieve a sustainable development, were coined. Sustainable development is defined as the development capable of satisfying today´s needs without compromising the capacity of future generations to satisfy their own needs. To achieve it, it is fundamental to harmonize three basic elements: economic growth, social inclusion and protection of the environment.

Anaerobic digestion (AD) of organic matter is a technology that can contribute to these basic elements of sustainable development. From an environmental perspective, AD provides a sustainable energy source, which is biogas or biomethane. It also provides a sustainable source of organic fertilizer which is the digestate (organic matter that remains after the anaerobic digestion). In the same way, it provides a powerful tool to mitigate methane emissions. And of course, AD is a sustainable waste management approach. The characteristics of biogas production are important for considering this approach as a promoter of circular economies, revalorizing waste from many sources.

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3.3.2 Energy

Introduction: Global energy demand has grown rapidly due to rising world populations and the consumerist lifestyle. Globally, energy consumption reached 1,5x10e14 kWh in 2010, and is estimated to be 2,34x10e14 kWh by 2040 (Sawatdeenarunat et al., 2015). Fossil fuels such as coal, oil and natural gas are the most used energy carriers providing more than 80% of global energy consumption (Sawatdeenarunat et al., 2015; Hijazi et al., 2016; Energy Statistics Pocketbook, 2022). These resources are limited in supply (non-renewable) and also have adverse effects on the environment due to the emission of greenhouse gases (GHGs) and particulate matter into the atmosphere, and also due to accidents that provoke leaks to the water and soil (US EIA, 2013). Unconvential fossil sources and new techniques, for example hydraulic fracturing, are becoming economically viable, but these alternatives entails even increased risks to the environment and reduced energetic yield (Hijazi et al., 2016).

According to the Fifth Assessment Report of the IPCC (Stocker et al., 2013), more than 50% of the observed increase in global mean surface temperature is very likely caused by the anthropogenic increase in GHG emissions, the main being CO_2. Fossil fuel sources (coal, oil and natural gas) account for 84% of total emissions of CO_2 (Friedlingstein et al., 2020),

Climate system and climate drivers: The climate system is the group of five components of the Earth: the atmosphere (air), the hydrosphere (water), cryosphere (ice and permafrost), litosphere (upper rock layer of the Earth) and biosphere (living organisms). Some authors consider the anthroposphere as a sixth component due to the significant effect of human actions over the climate system. These components interact by exchanges of energy and matter. The interaction determines what we define as the climate. The climate is the statistical description in terms of mean and variability of the relevant parameters of a specific place over a period, from months to thousands or millions of years. The parameters considered for the definition of climate are temperature, humidity, pressure, wind, and precipitations. A climate change refers to a change in the mean and/or variability of one (or more than one) of these parameters that persists for an extended period, generally decades or more (Stocker et al., 2013).

The climate system receives energy from the Sun (solar shortwave radiation, SWR) and, in much less quantity, energy from the Earth´s nucleus and energy of tidal waves from the Moon. Half of the SWR is absorbed by the Earth's surface, 20% is absorbed by the atmosphere, and 30% is reflected by the Earth's surface (albedo) and by aerosols, clouds, and gases in the atmosphere. The Earth surface also emits energy, which is in the form of longwave radiation (LWR, also termed infrared radiation). Changes in the outgoing LWR result from changes in the temperature of the surface or the atmosphere, or by changes in the emissivity of LWR from either the atmosphere or the Earth surface. The net balance between incoming and outgoing energies is called the energy budget of the Earth (Stocker et al., 2013).

Climatic parameters can change due to internal variability of the six components of the climate system, or by external substances or processes that affects the Earth´s energy budget.

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These so-called climate-change drivers can be natural or anthropogenic, and affects the climatic parameters by changing the energy (and matter) fluxes of the Earth. This is quantified by radiative forcing (RF). RF is a measure of the net change in the energy balance in response to an external perturbation. Positive values of RF means that the energy fluxes changed leading to warming of the Earth surface, while negative values of RF leads to surface cooling. For example, “atmospheric CO_2” is a climate driver. An increase in atmospheric CO_2 concentration leads to a positive RF, which means an increase in surface temperature. But, in the case of the climate driver “atmospheric aerosols”, an increase in atmospheric aerosol concentration leads to a negative RF, which means a decrease in surface temperature (Stocker et al., 2013).

Atmosphere, GHGs and carbon cycle: The atmosphere is the mass of gases that surrounds the Earth. It is mainly composed of diatomic nitrogen (N_2; 78%), diatomic oxygen (O_2; 21%), argon (Ar; 1%) and carbon dioxide (CO_2; 0.04%). Also, an important component is water vapor with a range from 0.1-5%. In a second place are neon (Ne; 18ppm), helium (He; 5ppm), krypton (Kr; 1ppm), diatomic hydrogen (H_2; 0.5ppm), xenon (Xe; 0.09ppm), methane (CH_4; 0.04ppm). In addition, there are other gases as traces (CO, N oxides, HNO_3, NH_3, H_2O_2, formaldehyde, CS_2, SO_2, O_3) (Baird and Cann, 2014). The atmosphere is divided altitude-wise into strata: troposphere (from sea level to 15km; 85% of the mass of the atmosphere), stratosphere (15km to 50km; here is the ozone layer), mesosphere (50km to 85km) and thermosphere (85km to 120km) (Baird and Cann, 2014).

The main climate-change drivers in the atmosphere are greenhouse gases, ozone, clouds and aerosols. They act affecting the radiative properties of the atmosphere absorbing incoming SWR emitted from the Sun or absorbing outgoing LWR emitted from the Earth’s surface. Absorption of SWR reduces the incoming heat, but absorption of LWR, after being reemitted in all directions, adds heat to the lower layers of the atmosphere and to the Earth. This is the greenhouse effect (Stocker et al., 2013).

As stated above, more than 50% of the increase in surface temperature can be explained by anthropogenic GHGs that accumulate in the atmosphere. The RF for the emissions of CO_2 from 1750 to 2011 is 2.29 W m^{-2}. The increase in the atmospheric concentration of CO_2 that resulted from anthropogenic emissions is the largest contributor of positive radiative forcing (Stocker et al., 2013).

Well-mixed GHGs represent the gaseous phase of global biogeochemical cycles, which can be defined as the complex flows and transformations of the chemical elements (and compounds containing that elements) between the different components of the climate system (atmosphere, ocean, land, ice, living beings) by biotic and abiotic processes. The carbon cycle accounts for the stocks and fluxes of carbon compounds, for example CO_2 and CH_4.

Carbon stocks are usually measured in relation to the mass of carbon atoms in the different carbonated compounds. In this way a common measure is a gigatonne of carbon (GtC) which equals a petagram of carbon (PgC) which equals 10e15 grams of carbon (Friedlingstein et al., 2020). In the atmosphere, the main carbon compound is CO_2 at a concentration of 390 ppm

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(corresponding to 828 PgC). Other carbon gases are methane CH_4 (3.7 PgC) and carbon monoxide CO (0.2 PgC), in addition to traces of hydrocarbons, carbon black aerosols and volatile organic compounds (Baird and Cann, 2014; Friedlingstein et al., 2020).

Carbonated compounds cycle between stocks which are located in all the components of the Earth. The exchange of carbon between different stocks is indicated as a quantity of carbon mass per year (i.e. GtC/yr or PgC/yr). Carbon compounds in the lithosphere and cryosphere are stocked in fossil reserves (gas, coal and oil), in the permafrost (frozen land) and in the soils; carbon compounds in the atmosphere are mostly stocked as CO_2 and CH_4, but also as other volatile compounds and aerosols; carbon compounds in the hydrosphere are stocked in the dissolved inorganic carbon, in organic compounds and in the ocean surface sediments; and carbon compounds in the biosphere are stocked in the marine or land biota (Stocker et al., 2013; Friedlingstein et al., 2020).

The carbon atoms are transferred from one stock to another. If the above-mentioned stocks are grouped in land (cryosphere, lithosphere and land biota), ocean (marine biota and hydrosphere) and atmosphere categories, and if only natural carbon fluxes are considered, there is a balanced efflux-influx of carbon between land-atmosphere, land-ocean and ocean-atmosphere groups. However, anthropogenic actions disturb this balance. Mostly by the huge extraction of carbon from fossil fuel reserves. The combustion of fossil fuels generates GHGs that are delivered to the atmosphere. The increased amount of CO_2 in the atmosphere leads to an increased uptake of CO_2 from the ocean. Within this situation, the net balance of carbon due to anthropogenic causes is -365±30 PgC/yr in fossil fuel reserves, +240±10 PgC/yr in the atmosphere and +155±30 PgC/yr in the ocean (Stocker et al., 2013). There are other carbon fluxes caused by anthropogenic actions but are not considered here due to the much lower impact in the carbon cycle.

Carbon stocks can be classified into two domains, defined by the amount and rate of carbon transfer to or from each stock. The fast domain is composed of stocks that exchange a high quantity of carbon compounds at a fast rate. The slow domain is composed of huge carbon deposits in rocks and sediments that are characterized by exchange times of more than 10k years. The natural carbon exchange between both domains is relatively small (less than 0.3 PgC/yr) and can be assumed to be constant over time. It is caused by volcanic emission events (CO_2), chemical weathering, erosion and sediment formation on the ocean floor (Stocker et al., 2013). Anthropogenic actions can be considered, in fact, to transform the fossil fuel reserves from a slow-domain stock into a fast-domain one, delivering high quantities of CO2 to the atmosphere. The use of fossil fuels since the industrial age has resulted in a huge transfer of carbon stocks accumulated in the slow domain to the fast domain, mainly in the atmosphere.

Environmental effects: The use of fossil fuels as a source of energy impacts the territories in many ways because of either the extraction, the transport, or the use of the fuels. The main and most dramatic impact is on the climate system, which entails effects over the five components of the climate system. The IPPC 5 Report describes the changes in temperature, the changes in energy budget and heat content, changes in circulation, changes in the water

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cycle and cryosphere, changes in sea level, changes in extremes, and changes in the biogeochemical cycles (Stocker et al., 2013).

The global mean surface temperature has certainly increased since the late 19th century, and with a steady increase in warming during for each of the last three decades, the 2000s decade has been the warmest. It is also virtually certain that globally the troposphere has warmed, and the stratosphere has cooled since the mid-20th century. The same high confidence is regarded to the virtually certain warming of the upper ocean (above 700m) since late 19th century. Improved sampling technologies also allows to state that likely the ocean from 700-2000m has warmed since 1957 to 2009, and that ocean from 3000m to the bottom has also warmed.

It is also virtually certain that the Earth has gained significant energy from 1971 to 2010, with more energy from the Sun entering to the climate system than exiting the atmosphere.

The atmospheric circulation (e.g. winds) and oceanic circulation (e.g. currents) have also suffered from changes. It is likely that atmospheric circulation features have moved poleward since 1970s, with a widening of the tropical belt, a poleward shift of storm tracks and jet streams, and a contraction of the northern polar vortex. The variability in major ocean circulation systems is also strongly evidenced, for example, it is very likely that the subtropical gyres in the North Pacific and Sout Pacific have expanded and strengthened since 1993.

The water cycle describes the continuous movement of water through the climate system in its liquid, solid and vapour forms, and storage in the reservoirs of ocean, cryosphere, land surface and atmosphere. The movement of fresh water between the atmosphere and the ocean can influence oceanic salinity, which is an important driver of the density and circulation of the ocean. It is very likely that regional trends have enhanced the mean contrasts in sea surface salinity since the 1950s: saline surface waters in the evaporation-dominated mid-latitudes have become more saline, while fresh surface waters in rainfall-dominated tropical and polar regions have become fresher. There is also very high confidence that the Arctic Sea ice extent decreased over the period 1979-2012. There is very high confidence that glaciers world-wide are persistently shrinking, while snow cover extent has decreased in the NH.

The increased uptake of heat by the oceans led to its warming which in turn led to the expansion of the ocean water and the increase in the Sea level. This is enhanced by the high transfer to the ocean of water currently stored on land, particularly from glaciers and ice sheets.

There is also evidence that climate change led to changes in extremes. For example, it is very likely that the number of cold days and nights has decreased, and the number of warm days and nights has increased globally between 1951 and 2010. It is also likely that since 1950 the number of heavy precipitation events over land has increased in more regions than it has decreased.

Finally, the global biogeochemical cycles have also suffered from changes due to effects related to climate system drivers. We will take about methane cycle in following headings.

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Opportunities for biogas industry: Even though the combustion of CH_4 molecules produces the same number of CO_2 molecules ($CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O$), the overall effect on global warming is smaller because methane effect as GHG is 28 times higher than CO_2 (Hijazi et al., 2016). Besides, the carbon source of biogas or biomethane belongs to a fast domain of the carbon cycle because it is generated from biomass that obtained the C atoms and compounds from the atmosphere (plants) or the biosphere (animals). So, contrary to fossil fuels, there is no net increase in CO_2 molecules in the atmosphere because the emitted CO_2 molecules are balanced to molecules that were transferred from the atmosphere to the biosphere.

The final version of the roadmap will take in consideration the results obtained over the course of MICRO4BIOGAS to analyse how our innovations in terms of new microbial communities can make this energy source more sustainable. For this, the improvements in yield, speed, and purity that we have set as goals will be of particular importance.

3.3.3 Artificial mineral fertilizers

Introduction: According to the Food and Agriculture Organization of the UN, the cultivated area worldwide was 4.8 billion hectares (ha) in 2019 (FAO, 2021). This agricultural land is used in two thirds as meadows and pastures, and one third as cropland. Although agricultural land decreased since 2000, on average there is a steady increase by 0,1% since 1961, with a peak in the 1990s. In spite of this, cropland area per capita decreased globally between 2000 and 2019 as population increased faster than cropland. This can be explained by an increase of 53% in primary crop production from 2000 to 2019 (9,4 billion tonnes in 2019).

However, this was not due to increased global workforce employed in agriculture. Indeed, this figure decreased from 1050 million people in 2000 to 874 million in 2020. On contrary, the increased production is explained by an increased yield due to developments in irrigation, increase in pesticide use (+36% from 2000 to 2019) and increase in fertilizer use (FAO, 2021). The same report states that the use of inorganic fertilizers worldwide in 2019 was 190 million tons of nutrients, of which 57% corresponds to nitrogen (N), while the rest corresponds to phosphorus (P) and potassium (K). This number represents an increase of 40% (54 million tons) of the fertilizer use since 2000, which is explained by the increase in fertilizer use per crop area, from 91 kg/ha in 2000 to 122 kg/ha in 2019, of which the N:P:K ratio is 70:28:24. Europe accounts for the 12% of the total fertilizer use. The main nutrient is N, representing 60% of the European fertilizers. Fertilizer use per area in Europe is 80kg/hectare, which represents a 12% increase since 2000 (FAO, 2021).

The nitrogen budget of the soil represents the difference between the amount of N added with synthetic and organic fertilizers and the amount of N taken away by livestock and crop production during their biomass growth. While a negative budget indicates that more N is being taken from the soil than added with fertilizers, which impacts yield and the health of the soils, a very positive budget indicates that excess fertilizers are being used, which leads to environmental and human health issues.

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Nitrogen cycle: While the fourth element in the biosphere is nitrogen, dinitrogen gas (N_2) is the main compound in the atmosphere. All N-containing species except N_2 are called reactive nitrogen species (Nr). These compounds are the N sources that support cellular metabolism and growth (Stein and Klotz, 2016).

The N cycle is composed by Nr compounds together with N_2, spanning nine different oxidation states of N atoms, which shows the complexity of this biochemical cycle. Roughly, there are three types of chemical reactions in the N cycle: N fixation, denitrification, and nitrification. N fixation is the reduction of N_2 gas to ammonia (NH_3) or ammonium (NH_4^+). These species are the only ones that can be assimilated by cells, which is when N interacts with C atoms making N organic compounds. Nitrifications are the reactions that oxidize NH_3 or NH_4^+ to nitrite (NO_2^-) or nitrate (NO_3^-). Denitrification are the reactions that transforms NO_2^- to less oxidized compounds, such as nitric oxide NO and nitrous oxide N_2O, and finally to the dinitrate gas N_2 (Stein and Klotz, 2016).

In the primordial atmosphere, abiotic reactions dominated the nitrogen cycle and provided oxidized forms of N (NO_2^- NO_3^-) and also reduced forms of N (NH_3 NH_4^+), the lasts being essential for biological assimilation (Stein and Klotz, 2016). Before the industrial age, the interchange between N_2 in the atmosphere to the rest of the Nr species was dominated by terrestrial and marine microorganisms via biological fixation of N_2 to ammonia, and in a much smaller extent, by NO_x production by lighting events (Fowler et al., 2013; Stocker et al., 2013).

Since the industrial era and specially since the 20th century´s Green Revolution, three anthropogenic sources of Nr have greatly increased Nr creation: the Haber-Bosch process to create NH_3 from atmospheric N_2 and H_2 for fertilizer and for industrial inputs; the cultivation of legumes and other crops capable of biological N fixation; and the combustion of fossil fuels, which converts atmospheric Nr from fossil fuels and N_2 into nitrogen oxides (NO_x) emitted into the atmosphere and redeposited on the land surface or in the oceans (Stocker et al., 2013; Stein and Klotz, 2016).

In 2010, global nitrogen fixation from atmospheric N_2 to terrestrial and marine ecosystems was 413 Tg N as estimated by Fowler et al. (2013). Of this Nr, 210 Tg N were fixed by anthropogenic causes, either intentionally or unintentionally. The main anthropogenic causes were the fertilizer production through Haber-Bosch process (120 TgN) and agricultural fixation by legumes and grasses (60 TgN), with 30 TgN produced unintentionally by combustion. The main natural cause was the marine biological fixation of 140 TgN, followed by terrestrial biological fixation of 58 TgN and 5 TgN fixed by lightning (Fowler et al., 2013).

The Nr that is denitrified to N_2 is between 100-280 TgN/yr depending on different authors. The remaining N (difference between fixed and denitrified) is: emitted as NO or N_2O from soils to the atmosphere (5 and 13 TgN/yr respect.) or as N_2O from the oceans to the atmosphere (5.5 TgN/yr); emitted as NH_3 from terrestrial ecosystems and oceans to the atmosphere (60 and 9 TgN/yr respect.); wet and dry deposited as NO_x to terrestrial surfaces and oceans (70 and 30 TgN/yr); and buried in oceans (20 TgN/yr) (Fowler et al., 2013).

The concept of nitrogen cascade illustrates how an atom of Nr circulates along the biogeochemical pathway, with different effects to the environment and human health, until it is denitrified again to nonreactive N_2. For example, a N cascade can start with the use of fertilizer, transforming non-reactive N from atmospheric N_2 molecules to Nr (NH_3) by Haber-

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Bosch process. From the 120 TgN fixed by Haber-Bosch, 80% is used as agricultural fertilizer and 20% as feedstock for industrial processes. Half the Nr fertilizer applied to agroecosystems is incorporated into crops harvested for human and livestock consumption. The other half is transferred to the atmosphere as NH_3, NO, N_2O or N_2, or deposited as NO_x to terrestrial surfaces and oceans (Fowler et al., 2013).

Environmental effects: As stated above, when Nr creation rates are higher than the rates of removal by denitrification or assimilation by cell metabolism, the remaining Nr is available in the atmosphere and in marine and terrestrial ecosystems, and is either accumulated or dispersed by hydrologic (leaching and runoff) and atmospheric transport processes. (Galloway et al., 2003; Erisman et al., 2013).

The environmental effects of the excess of Nr are varied. Emissions of N_2O are the third GHG source in importance, together with CO_2 and CH_4; the global balance of the effect of Nr on terrestrial radiation, this is in global warming, is 0.24 Wm_{-2}. N_2O also degrades the stratospheric ozone layer. NO_x species produce tropospheric O3 and nitrate aerosols increasing smog and the haziness of the troposphere. NH3 leads to the production of nitrate aerosols. Together with S, Nr contributes to the acidification of lakes, rivers, and streams, followed by loss of biodiversity. Nr increases productivity in forests, grasslands, and waters, which can lead to eutrophication and reduced biodiversity. In coastal ecosystems, Nr is considered one of the biggest pollution problems, again leading to eutrophication, hypoxia, and loss of biodiversity. Increased soil Nr changes the rate of decomposition of soil organic matter and therefore affects the emission of CO_2; it also affects plant productivity, either increasing it because of the greater availability of Nr, but also decreasing plant availability due to volatile organic compounds and Nox-mediated tropospheric O_3. Increased NO_x, aerosols, tropospheric O_3, and nitrates in drinking water also negatively impact human health provoking respiratory illnesses, cancer and cardiac diseases (Galloway et al., 2003; Leach et al., 2012; Stocker et al., 2013).

Opportunities for biogas industry: Nitrous oxide (N_2O) emissions from fertilizer application and manure management account for 5% of total Europe GHG emissions (210 $MtCO_{2eq}$) (Bacenetti et al., 2013). Although Europe is the only region where the N soil budget declined (by 5%) from 2000 and 2018, it is still considerably high, with a budget of 48kg/ha. This budget was made from an input of 90.7kg/ha of N and an output of 42.9kg/ha. Of these 90.7 kg/ha of input, 51.4 kg/ha correspond to synthetic fertilizers, 24.8 kg/ha correspond to manure, and 0.7 and 4.8 kg/ha correspond to atmospheric deposition and biological fixation respectively (FAO, 2021).

The organic agriculture differs from conventional agriculture in that it promotes the avoidance of synthetic fertilizers and pesticides. Fifteen of the top 20 countries with the highest ratio of organic:conventional agriculture is in Europe. This means that the region has emphasized the importance of organic agriculture. However, the percentage of organic agriculture from the total agricultural area is still low with a 3,4% in Europe (FAO, 2021).

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Digestate as a by-product from biogas production via AD can substitute synthetic fertilizer and be used as an organic fertilizer to boost the adoption of organic agriculture practices in Europe (Hijazi et al., 2016).

However, there are many things that must be addressed before adopting digestate as a fertilizer. For example, the digestate from animal manure may contain high concentrations of elements such as copper and zinc (micro-nutrients supplemented in animal feed), and direct application of the digestate to agricultural land may result in phytotoxicity. Under such conditions, the digestate must be diluted with irrigation water before being applied to the field (Sawatdeenarunat et al., 2015). Also, open storage of digested residues is considered a hot spot of emissions in biogas systems. If the CH_4 produced during digestate storage is not properly recovered, thus the released CH_4 may worsen GHG emissions (Sawatdeenarunat et al., 2015; Hijazi et al., 2016).

For the final version of the roadmap we will assess the composition of the digestate that will be produced by microbial communities developed by MICRO4BIOGAS. We will compare this digestate with non-improved microbial communities to analyse if our communities can generate a better and sustainable organic fertilizer.

3.3.4 Methane emissions

Introduction: Climate warming is a global phenomenon caused mainly by the abundance of well-mixed GHGs in the atmosphere. Among the GHGs, the most important is CO_2. However, methane emissions (CH_4) have a significant contribution to global warming. Methane, as well as CO_2, are compounds that are integrated into the so-called carbon cycle above explained. At normal pressure and temperature conditions, CH_4 is a gaseous compound, so most of it is located at the atmosphere. There, the concentration depends on the ratio among sources:sinks.

Methane cycle: In the methane cycle, there are much fewer sinks than sources. Indeed, 90% of atmospheric CH_4 is eliminated by the oxidation with hidroxil radicals (OH), mostly at the troposphere. The OH radicals are made after the photolysis of O_3 in the presence of water vapour (and are then eliminated by reaction with CO, CH_4 and non-methane volatile organic compounds). The remaining methane is lost by photochemistry in the stratosphere (reaction with atomic clorine (Cl), atomic fluorine (Fl) and excited atomic oxigen (O(1D))), by photochemistry in the marine boundary layer and by oxidation in soils. This last methane sink is due to methanotrophic bacteria that can oxidize and consume methane as a source of energy in unsaturated oxic soils (Saunois et al., 2020).

As stated above, there are many sources of CH_4. They can be classified based on the process that creates CH_4, or based on if the process is provoked by natural or anthropogenic causes. The processes that can create CH_4 can be biogenic, geological, or pyrogenic. Biogenic methane is a result of the anaerobic digestion of organic matter performed by communities of microorganisms. Geological processes that emit CH_4 are for example volcanic eruptions, gas

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reserves, permafrost or methane hydrates (accumulated in the ocean) that emits CH_4. Pyrogenic processes emits CH_4 because of the incomplete combustion of fossil fuels or biomass (Stocker et al., 2013).

Combining both of the classifications, Saunois et al 2020 describe three anthropogenic categories: (1) agriculture and waste, (2) fossil fuels, and (3) biomass burning and biofuels; and eight natural categories: (1) wetlands, (2) other inland water systems, (3) onshore and offshore geological sources, (4) termites, (5) wild animals, (6) oceanic sources, (7) terrestrial permafrost and hydrates, and (8) vegetation.

The anthropogenic fossil fuel related methane emissions are due to the exploitation, transportation and usage of coal, oil and natural gas. The composition of natural gas is mostly methane. The extraction, transportation or use of gas can all contribute to methane emissions. The same accounts for shale gas, which emits in a similar amount as natural gas. Also, for example in coal mines, high quantities of CH_4 are pumped out to maintain a CH_4 percentage of 0,5% inside the mine. While in some countries this CH_4 is used as fuel, in many countries it is still emitted to the atmosphere. However, the highest source of anthropogenic CH4 is agricultural and waste, mainly by livestock production, and followed by rice cultivation, landfills, and wastewater treatment. The livestock emissions are due to domestic ruminants whose digestive system, particularly the rumen, provides methanogenic Archaea with stable temperatures (39ºC), optimum pH (6.5-6.8) and a constant plant matter flow. These Archaea produce methane that is released mostly through the mouth of the animals (87%) or absorbed in the blood system. The rest of the CH_4 is emitted through the rectum of ruminants. Manure decomposition is another important source of CH_4 when manure is treated in liquid or slurry forms. If manure is deposited as a solid, aerobic conditions produce no CH_4, but it produces N_2O which has a larger warming impact than CH_4. Flooded fields for rice cultivation are another important source of anthropogenic CH_4. Finally, landfills and wastewater handling produce CH_4 according mostly to the type of waste and to the amount of degradable organic material respectively. In some landfills, however, CH_4 is used to generate heat instead of just ventilating it to the air. And for example, Europe counts with laws for the regulation of landfilling. Finally, biomass and biofuel CH_4-emissions are 90% produced by anthropogenic causes compared to a 10% of natural fires (Stocker et al., 2013; Saunois et al., 2020).

The natural sources of CH_4 are even more varied, as stated above. Wetlands are ecosystems where soils or peats are saturated with water, or where surface inundation dominates the soil biogeochemistry. The anaerobic conditions lead to CH_4 production. Other inland water systems can also produce CH_4, such as lakes, ponds, reservoirs, streams, or rivers. Also, there are important onshore and offshore geological sources of CH_4 emissions produced in the Earth crust that reach the atmosphere through tectonic faults and fractured rocks. Just like livestock, wild ruminants also emit CH_4 when they degrade plant biomass in their digestive system through anaerobic digestion. There are even important emissions from insects that generate CH_4 in their guts. The most representative are the termites. There are also oceanic sources of CH_4, both in coasts and in open ocean. For example, CH_4 production from marine sediments, in situ production in the water column, leaks from geological marine seepage and also emission from the destabilization of marine hydrates. Marine CH_4 hydrates are ice-like crystals formed under specific temperature and pressure conditions that comprise a potential important source of CH_4, but without a significant relevance yet. The permafrost,

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which is frozen soil below 0ºC, can generate direct and indirect CH_4 emissions. The direct ones are due to the release of CH_4 contained in thawing permafrost, which was created before the last glacial era and was then trapped as temperatures went down. Indirect emissions rely on methanogenesis induced when the organic matter is released from thawing permafrost. This source is projected to be more important as warming climate thaws more permafrost. Finally, vegetation can produce small amounts of CH_4, either through abiotic photochemical processes induced by stress, acting like straws releasing CH_4 from soils, or providing suitable environments for methanogenic microorganisms, especially in the stems (Stocker et al., 2013; Saunois et al., 2020).

Since 1750 (beginning of industrial era) to 2011, atmospheric CH4 levels grew exponentially by a factor of 2.5, from 0.7ppm to 1.8ppm. The 5th intergovernmental panel for climate change stated, with a very high statistical confidence level, that this increase was due to anthropogenic causes. In fact, satellite measurements show that there are higher CH_4 concentrations in places where anthropogenic influence is high, for example in urbanized or agricultural areas. This effect can also be detected downwind of urbanized or agricultural areas (Stocker et al., 2013). Actual measurements indicate that anthropogenic causes explain 50-65% of total emissions. And top-down approaches estimates that for the period 2008-2017, there was an atmospheric growth of 18.2 $TgCH_4$/yr (Stocker et al., 2013; Saunois et al., 2020).

Environmental effects: Although exposure of hydrocarbon mixtures can have some adverse effects on humans, methane emissions are not considered relevant for direct health issues. However, the global warming potential of CH_4 as a GHG is estimated to be 28-36 times higher than CO_2 in a 100 year lapse. In this way, it is the second major component among anthropogenic GHG (Paolini et al., 2018).

The RF associated to CH_4 emissions are estimated to $0.97Wm_{-2}$, while for the same period, total GHG and CO_2 RF are $3Wm_{-2}$ and $1.68Wm_{-2}$ respectively. This reveals the considerably high effect of CH_4 as GHG (Stocker et al., 2013). Almost a quarter of the RF associated to CH_4 is not due to a direct consequence of CH_4 itself, but due to its reaction with O2 that produces OH radicals and then water. Stratospheric water vapour also acts as a GHG (Stocker et al., 2013).

In Europe, CH_4 emissions from enteric fermentation, manure management and rice cultivation produces 5% of total Europe GHG emissions (195 $MtCO_{2eq}$) (Bacenetti et al., 2013).

Opportunities for biogas industry: Biogas production avoids the emission of CH_4 to the atmosphere from organic matter going through anaerobic digestion. However, there remains spots of avoidable emissions. For example, the open storage of not-fully digested residues or flaring excess biogas (Hijazi et al., 2016).

MICRO4BIOGAS plans to develop new designs of bioreactors for an improved AD of organic matter. The final version of this roadmap will assess these designs to identify if the emissions of CH_4 will be reduced compared to current bioreactors.

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3.3.5 Waste

Introduction: Wikipedia defines waste as "*unwanted or unusable materials; any substance which is discarded after primary use, or is worthless, defective and of no use*". The European Union defines waste as "*an object the holder discards, intends to discard or is required to discard*". There are many types of waste, each one with also many forms to be managed. Some types of waste are agricultural waste, chemical waste, construction and demolition waste, food waste, green waste, wastewater, sewage, organic municipal waste, etc.

If we focus on municipal waste, annually 2 billion tons of this waste are globally produced. And this is without considering the two other components of organic solid waste, which are agricultural waste and animal waste (Wainaina et al., 2020). The EU countries contribute with approximately 250 millions of tons of municipal solid waste, which leads to an estimate of 482kg per capita (Bourguignon, 2018; Commission, 2020). This represents 10% of total waste generated in Europe (Bourguignon, 2018).

The level of waste generation on EU countries positively correlates with the gross domestic product per capita, which means that the amount of waste depends significantly on economic development. However, the increasing waste production in EU countries does not correlate with an increase in the adoption of reducing- or reusing-behaviours. This is different for the recycling behaviour, which correlated with the amount of waste generated, showing to be the only positive-behaviour adopted when people try to manage their waste production. So, as standard of living rises, it also does the level of consumption and the amount of waste, which has to be managed properly (Minelgaitė and Liobikienė, 2019).

AD with different residues: Manure as feedstock is a very favourable substrate in terms of environmental impact. Biogas scenarios with manure as input material show lower GHG emissions than their reference systems. The transportation of manure can increase the impact of this feedstock (Hijazi et al., 2016). Animal manure shows more environmental benefits than wastes from food industries and households, with an indirect benefit because of avoided emissions of CH_4 and N_2O from traditional manure storage. Also, the manure digestion can be even improved for an increased methane yield if animals are fed with higher crude protein content food (Hijazi et al., 2016).

Manure is a good substrate to be used in co-digestion treatments. For example, co-digestion of animal manure with lignocellulosic residues can be used to biologically pretreat energy crops and remove the amorphous hemicellulose fraction of the biomass structure (Sawatdeenarunat et al., 2015). The codigestion of energy crops with manure is beneficial for the AD because it increases the yield of biogas, stabilizes the organic matter and decreases the methane emission during storage (Bacenetti et al., 2013).

Wastewater treatment is an energy intensive activity. The treatment of municipal waste-water accounts for about 3% of global electricity consumption and 5% of global greenhouse gas emission. The biological wastewater treatment demands 20-30 kWh energy per person equivalent per year, but the energy recovery via AD of wastewater sludge is only about 15–18 kWh per person equivalent per year. So, utilizing the sludge for energy recovery, WWTP can achieve energy self-sufficiency only up to approx.. 65%. In practice, a typical WWTP can

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currently offset 20–30% of the energy consumption and greenhouse gas emission (Nghiem et al., 2017).

Traditionally, wastewater treatment plants (WWTPs) have been designed with the aim of meeting discharge standards to receiving water bodies while waste produced during treatment is destined for landfill or incineration.

An option to make WWTPs more convenient both economically and ecologically is the co-digestion of wastewater sludge with substrates with enough stock availability. For example, wastewater sludge may be codigested with municipal organic waste. With this, WWTPs could achieve energy-neutrality and reduce the cost of municipal organic waste management while facilitating nutrient recycling. Other possible substrates for this may be food waste from urban areas, organic waste from food processing, market waste, dairy waste, etc (Nghiem et al., 2017).

Codigestion is also a viable alternative for small WWTPs that require a specific biogas production threshold that can justify the maintenance cost and capital investment of biogas utilization equipment such as combined heat and power unit (Nghiem et al., 2017).

It is estimated that in EU countries, 20% of the food produced is lost (Commission, 2020). Food waste can be sorted and processed either onsite or delivered in a pre-treated liquid form. In addition to sorting and processing equipment, onsite processing also requires several auxiliary facilities including weighing bridge and even airlock passage to the receiving bay (Nghiem et al., 2017).

<u>Environmental effects</u>: The collection of OSW is an environmental concern both in urban and rural areas. Technologies for OSW management are important to tackle environmental issues, to develop sustainable practices, and to forge circular economies. Currently, most of the OSW is treated to be converted in organic fertilizers, or is deposited in landfills or incinerated (Wainaina et al., 2020).

EU countries, just like the rest of the countries in the world, have produced increasing masses of wastes for many decades. The most practical solution for waste management in most of EU countries remains landfilling due to technical, economic, and legal reasons. This is true even with the European Union Directives on waste landfills that has introduced specific goals for reducing the volume of disposed waste and strict requirements of landfilling and landfill sites (Vaverková, 2019).

Landfilling is the worst option in environmental terms: it provokes contamination on soil and water with chemicals that leach from waste, animals can ingest microplastics, and methane and other pollutants are released to the air. Also, in economic terms, landfilling leads to the loss of valuable resources that could be used for manufacturing other goods (Bourguignon, 2018).

Waste disposal options by landfilling or incineration are expensive and not compatible with the concept of a circular economy. Ongoing leachate management and extensive monitoring for potential groundwater and air pollution are required during active landfill operation and

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even up to 50 years post-closure. On the other hand, extensive air pollution control is required for the waste incineration. Incineration of waste materials also results in significant greenhouse gas emission (Nghiem et al., 2017).

Sharma et al., (2019) recognizes the positive environmental effects for the recycling (composting, vermicomposting and AD) of agricultural organic wastes and the use of the treated waste (e.g. digestate): improved soil texture and fertility, enhanced crop productivity, GHG´s mitigation and availability of alternatives to agrochemicals.

Opportunities for biogas industry: Potentially, 60 million tons of OSW could be recycled by AD and composting technologies in Europe, which could save one million tons of nitrogen and 20 million tons of organic carbon that are currently lost through landfilling organic waste (Mayer et al., 2019). European countries in average recycle only 5% of the total OSW (Commission, 2020). If a higher portion of OSWs could be recycled and reused, it is estimated that approximately 30% of the chemical fertilizer applied to soil could be replaced (Paes et al., 2019).

EU-28 nations produced nearly 25.38 million tons of wastes through all the activities including economic and households in 2016. It is estimated that global urban waste collection market can reach to an approx. income of U$S 410 billion. Only 25% of this waste is recycled, which means that there is a big economic opportunity (Wainaina et al., 2020).

While policy efforts do not seem to succeed in changing personal habits regarding the reduction of waste, AD of municipal waste may be an important tool for the waste management. This is further relevant considering the close link among economic development and waste production.

A study that analysed national and regional approaches linked to circular economy models in several European countries identified waste management strategies in almost every of these approaches, and concluded that waste management appears to be critical in this transition (Vanhamaki et al., 2019).

In the final version of this roadmap, we will assess the extent to which AD of organic waste could tackle the currents issues regarding waste management. And we will also assess the efficiency of microbial communities developed by MICRO4BIOGAS for degrading different types of feedstocks obtained by several sources of waste.

3.3.6 Economical aspects of biogas industry

Introduction: In the previous sections it has been analysed the environmental potential of biogas and digestate production by AD. It has been summarized in which way this technology can provide sustainable alternatives for waste treatment, energy production and land fertilizing. However, the environmental challenges also affect the global economy in several ways. And news approaches arise to meet not only environmental goals, but also increasing the profits and decreasing the loses in terms of money.

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Two important concepts that emerge as pillars of a sustainable economy are (1) circular economy and (2) bioeconomy/biorefineries. These concepts will be explained in the following sections. However, in sum, they relate to the biogas production because AD can be a pivotal piece for the development of circular economies, valorising waste from different sources; and in the way that AD can be considered as a bioeconomy process, obtaining energy and fertilizer without appealing to fossil-derived resources.

Circular economy: Circular economy is defined as an economic model where the value of products and materials that they contain are valued for as long as possible. It differentiates from the linear economy based on the "take-make-use-dispose" pattern, and considers waste as another resource that can be reintroduced to the production cycle (Bourguignon, 2018; Vanhamaki et al., 2019). The aim of circular economy is the maximization of resource efficiency and minimization of waste production, with the concomitant effects of keeping resource consumption within planetary boundaries and reducing production costs.

Obsolete products or waste materials are turned into resources for another purpose, thus closing loops and minimizing waste. By adopting circular economy strategies, several European countries are estimated to be able to reduce their national GHG emissions by 70%, while growing their workforce by 4% and decreasing their dependency from resource and energy import (Nghiem et al., 2017).

Two important concepts related to circular economy and waste management are the extended producer responsibility (EPR) and the waste hierarchy (Bourguignon, 2018). The EPR states that producers have the responsibility for collecting used goods as well as sorting and treatment for recycling. The waste hierarchy implies that waste may be treated preferentially by prevention, then by preparing for re-use, then by recycling, then by energy recovery and finally disposal.

Bioeconomy and biorefineries: The European Commission defines bioeconomy as "*the production of renewable biological resources and the conversion of these resources and waste streams into value added products, such as food, feed, bio-based products and bioenergy*". While some people is strongly optimistic on the potential positive effects of this way of production, others argue that bioeconomy could perpetuate the linear economy model (Stegmann et al., 2020).

In this way, the European Commission stated that bioeconomy should be coupled to sustainability and circular economy, coining the concept of circular bioeconomy. In turn, the bio-based circular economy concept refers specifically to the reuse and valorisation of organic wastes and residues (Wainaina et al., 2020). Other options of waste management such as landfilling, and incineration offer very limited possibilities for resource recovery. But in a bio-based circular economy, organic wastes represent a convenient source of resources in terms of energy and nutrients (Nghiem et al., 2017).

A biorefinery system can be defined as the processing of non-fossil-based feedstock (biomass/organic waste) through biological or chemical unit operations into a wide range of marketable products (energy, materials, and chemicals). Biorefineries can utilize while valorising

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a diverse range of renewable feedstocks such as biomass from forestry, agriculture, aquaculture, and waste (agricultural and biogenic municipal waste) (Katakojwala and Mohan, 2021).

The classification of biorefineries are based on (1) the type of feedstock (biomass, waste, gas, etc.), (2) the biocatalyst-organism (bacteria, yeast, fungi, algae, etc.), (3) the type of process/technology (fermentation, acidogenesis, methanogenesis, photosynthesis, thermochemical, catalysis, etc.), and (4) the targeted product(s) or generation (first, second, third and fourth) (Katakojwala and Mohan, 2021).

The major challenges of biorefineries are its acceptance in the existing fossil-based market, the feedstock availability and composition, the volumes to meet market requirement, the resource recovery efficiency, the techno-economic feasibility, and the environmental sustainability. Taking a biorefinery to a commercial scale is an intricate issue because the biobased products face severe competition from petro-chemicals with respect to their market cost, and therefore, consistent processes and competitive products compared to conventional counter parts are needed. Biorefineries shouldn´t only be assessed for their economic competitiveness, but also for their environmental sustainability. Life-cycle assessments and techno-economic analysis (TEA) can help with this (Katakojwala and Mohan, 2021).

Although AD is a widely adopted technique for the obtention of renewable bioenergy, biogas production alone may not sufficiently justify the capital and operational costs. More likely, AD can be integrated into a biorefinery process (Sawatdeenarunat et al., 2015).

The main by-product obtained with biogas is biomethane. This is made by the upgrading of biogas with different techniques as stated in Section. Once converted in biomethane, this product may be used to feed the gas system or as vehicle fuel. The conversion of biogas to transport fuel has recently been implemented in several EU countries including Germany, Italy, and Sweden (Nghiem et al., 2017).

The consortium of microorganisms in the AD of lignocellulosic matter can easily convert the sugar constituents of hemicellulose into CH4. Recalcitrant lignin and cellulosic fibres stays in the digested residue, which can be treated with hydrolytic enzymes to solubilize monomers that can be used as precursors in the production of diverse products ranging from bioenergy/biofuel (i.e., CH_4,H_2, ethanol and butanol)) to organic acids (e.g., succinic acid) and biopolymers (e.g., bioplastic) (Sawatdeenarunat et al., 2015). Successful production of bioplastic from biogas has been demonstrated at proof-of-concept experimental levels (Nghiem et al., 2017).

Regarding to the effluent of lignocellulosic AD, it may be combined with microalgae production as a nitrogen rich feedstock that may be utilized by algae as a nutrient source. Such produced algae could be utilized for biodiesel production and the residue after lipid harvest could be further fed into the digester for CH_4 production (Sawatdeenarunat et al., 2015).

Incentives: According to (Raboni and Urbini, 2014) the incentives granted to the producers and users of biogas and biomethane are in general of the following kinds:

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- Renewables Obligations that support renewable electricity generation by requiring suppliers to increase the generators production through the purchase of tradable Renewable Obligation Certificates.
- Feed-in Tariffs which comprises an extra payment for renewable electricity producers, mainly addressed to support small generation plants.
- Renewable Heat Incentive, which provides a guaranteed payment for heat used from biogas combustion and all the biomethane injected into the grid of natural gas.
- Renewable Transport Fuels Obligation which places an obligation on fuel suppliers to source 5% of their transport fuels from renewable sources by 2014.
- Various incentives and tax breaks introduced in a few countries to promote the use of biomethane in transport.

In the 2010s, there was an expansion of AD plants in Europe mostly thanks to feed-in-tariffs scheme in 29 countries (Bacenetti et al., 2016). These incentives aims an economical feasibility of biogas plants at electricity markets (Saracevic et al., 2019).

<u>Quantitative analysis of economic impacts</u>: The economic analysis of AD plants can be evaluated using different indexes. For example, the net present value (NPV) is operationalized by calculating the costs of the plants (negative cash flows) and the plant cash inflows (positive cash flows) for each period considered. The payback period is defined as the time span required to recover the cost of a project or an investment. And the internal rate of return is the discount rate that makes the net present value of an investment zero (Lovarelli et al., 2019).

However, the technique most widely used to analyse economic yields of AD plants are the technoeconomic analysis. These assessments can help to predict an economically competitive production process of bio-based products compared to petro-chemical derived products (Katakojwala and Mohan, 2021). During the techno-economic analysis, three areas of anaerobic digestion process are important: (1) unit operations with collection and transport of feed stocks, (2) to provide the treatment facility for production of biogas, and (3) upgrading the bio-energy for various applications as electricity and liquid methane for household cooking (Saracevic et al., 2019).

<u>Opportunities for biogas industry</u>: The reduction of capital and operating costs of AD facilities is promoting the growth of the biogas industry. It is estimated that the cost of production of biogas will reduce 38% by the year 2050 compared to 2015. It the estimated that biogas production worldwide had an average growth rate of 11.2% reaching up to 58.7 billion Nm3 in 2017 (Wainaina et al., 2020).

The biogas market is estimated to reach 50 billion in 2026 (Waste Management World 2017). It is estimated that only from the global urban waste collection market, the income through the AD of this waste can be around 410 billion incomes. However, currently only 25% is recycled (Wainaina et al., 2020).

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In the final version of the roadmap, we will further analyse how the improvements that we propose as goal for MICRO4BIOGAS could

3.3.7 Economical aspects of biogas industry

3.3.7.1 Life cycle assessment (LCA)

Introduction: The AD of organic matter is an activity that has many implications in environmental terms, as stated above. However, as any other economic activity, it is important to analyse thoroughly the possible impacts that it may provoke on the environment, either positive or negative.

There are different ways to quantitatively analyse the environmental impact of the AD of organic matter. One of the most used is the so-called Life Cycle Assessment (LCA). LCAs systematically measure the effect of an economic activity based on different environmental parameters. The main focus is the measurement of the resources and energy consumed and the waste emitted into the environment (Hijazi et al., 2016). The analysis contemplates the entire life cycle of a product, from the acquisition of the raw material to the recycling or disposal of the product or the waste of the economic activity (Bacenetti et al., 2013).

Other techniques are also used for the estimation of environmental impact: environmental performance evaluation, environmental impact assessment, risk assessment, etc. (Wang and Liu, 2021). However, two characteristics identify LCA and differentiate it from the previous: LCAs are relative and comparative.

The LCAs are relative to a functional unit. The functional unit is used as a quantitative measure to normalize the data describing the functioning of the system in terms of energy and matter that is used and that is produced (Ingrao et al., 2019). For example, a LCA of a car factory may define one car as the functional unit. In this case, inputs and outputs consumed and produced by the system during the fabrication of one car will be measured.

The LCAs are comparative studies where the environmental burden of two or more scenarios are calculated. Each scenario is assigned a value for each environmental impact category. The overall impact can be used to choose the best option under study (Timonen et al., 2019). In the example of the car factory, considering the same functional unit as above, an LCA could compare a scenario where the entire factory pipeline is managed by humans against another scenario with robot-assisted phases.

Although there is not a unique way to perform an LCA, the general protocol is defined by international standards (ISO 14040). An LCA must adhere to these standards to be compared to other LCAs. According to them, each study comprises four phases: goal and scope definition, Life Cycle Inventory, Life Cycle Inventory Analysis, and final phase of interpretation.

First phase: Goal and scope: The first phase of an LCA is where the goal and the scope are defined. Together with the scope, there may also be expressed the reasons for the making of the assessment and the target audience. The scope of the assessment involves the definition of both the functional unit and the boundaries of the productive system.

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The definition of the system boundaries means the explicit expression of all the stages that will be considered as part of the activity that will be assessed. Each stage is connected to each other by fluxes of energy, intermediate products, or waste to be treated. For example, the product of one stage may be the substrate for the following unit. Dividing the system in these units facilitates the identification of resource/energy entry points to the system, and waste or products exit points.

The system boundary specification and the stage of a process leads to the definition of the unit processes of the system intended for the study, which act as key-element in assessing the sustainability of a product/process. The system boundaries can be considered from any segment of the product chain [Gate-to-Gate, Cradle-to-Gate, Cradle-to-Grave, Cradle-to-Cradle] to include various stages like biomass cultivation, transport, processing of biomass and products manufacture in biorefinery, transport of products to the consumers, customer use of the products, and product disposal to the environment (Katakojwala and Mohan, 2021).

Second phase: Life Cycle Inventory: The second phase is called the Life Cycle Inventory (LCI). All the data corresponding to input or output spots of matter or energy is compiled for each unit of production (Hijazi et al., 2016). The data may be classified according to headings like (1) resource inputs, (2) waste outputs, (3) other outputs (Table 24).:

Table 24: *Classification of LCI data extracted from https://ecochain.com/knowledge/impact-categories-lca/*

	Parameter	Unit	Description
Resource inputs			
	Primary renewable energy (materials)	MJ	Use of renewable primary energy resources as raw materials
	Primary renewable energy (energy)	MJ	Use of renewable primary energy, excluding renewable primary energy resources used as raw materials
	Primary renewable energy (total)	MJ	Sum of the two values above
	Primary non-renewable energy (materials)	MJ	Use of non-renewable primary energy resources as raw materials
	Primary non-renewable energy (energy)	MJ	Use of non-renewable primary energy, excluding renewable primary energy resources used as raw materials

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	Primary non-renewable energy (total)	MJ	Sum of the two values above
	Use of secondary material	kg	Material recovered from previous use or from waste which substitutes primary materials
	Use of fresh water	m3	Freshwater use in absolute values
	Use of renewable secondary fuels	MJ	Renewable fuel recovered from previous use or from waste which substitutes primary fuels
	Use of non-renewable secondary fuels	MJ	Non-renewable fuel recovered from previous use or from waste which substitutes primary fuels
Waste outputs			
	Hazardous waste disposed	kg	Hazardous waste has a certain degree of toxicity that necessitates special treatment
	Non-hazardous waste disposed	kg	Non-hazardous waste is non-toxic and similar to household waste. It consists of inert waste and ordinary household waste
	Radioactive waste disposed	kg	Radioactive waste mainly originates from nuclear energy reactors
Other outputs			
	Components for re-use	kg	Material or components leaving the modelled system boundary which is destined for reuse
	Materials for recycling	kg	Material leaving the modelled system boundary which is destined for recycling
	Materials for energy recovery	kg	Material leaving the modelled system boundary which is destined for use in power stations using secondary fuels.
	Energy production	MJ	Energy exported from waste incineration and landfill

<u>Third phase: Life Cycle Impact Assessment (LCIA):</u> The third phase is the Life Cycle Impact Assessment (LCIA). The data of the LCI is analyzed in terms of impact categories to assess which is the effect of each scenario under study over the environment.

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The quantification of the impact for any input or output is based on scientific evidence. For example, in the case of Green House Gases (GHGs) CO_2, CH_4 and N_2O, the estimation of the effect of each gas under the Global Warming Potential category is performed normalizing the effect of each amount of gas to the equivalent amount of CO2, the standard for this category. For example, an amount of N_2O equals 265 amounts of CO_2, and an amount of CH_4 equals 28 amounts of CO_2 (Hijazi et al., 2016).

There are different categories where data from the LCI can be allocated for the impact assessment. Selection of appropriate impact categories that specifically fit the process is significant (Katakojwala and Mohan, 2021; Table 25). The preferred way to evaluate the energy inputs is to integrate various kinds of impact categories to a solitary weighted environmental impact metrics (much like endpoint categories) by employing characterization, weighing and normalization factors which assists to convert emissions and resource consumption into impact categories (Katakojwala and Mohan, 2021).

Table 25: List of impact categories https://ecochain.com/knowledge/impact-categories-lca/

Impact category	Unit	Description
Climate change – total, fossil, biogenic and land use	kg CO_2-eq	Indicator of potential global warming due to emissions of greenhouse gases to air. Divided into 3 subcategories based on the emission source: (1) fossil resources, (2) bio-based resources and (3) land use change.
Ozone depletion	kg CFC-11-eq	Indicator of emissions to air that cause the destruction of the stratospheric ozone layer
Acidification	kg mol H^+	Indicator of the potential acidification of soils and water due to the release of gases such as nitrogen oxides and sulphur oxides
Eutrophication – freshwater	kg PO_4-eq	indicator of the enrichment of the fresh water ecosystem with nutritional elements, due to the emission of nitrogen or phosphor containing compounds
Eutrophication – marine	Kg N-eq	Indicator of the enrichment of the marine ecosystem with nutritional elements, due to the emission of nitrogen containing compounds.

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Eutrophication – terrestrial	mol N-eq	Indicator of the enrichment of the terrestrial ecosystem with nutritional elements, due to the emission of nitrogen containing compounds.
Photochemical ozone formation	kg NMVOC-eq	Indicator of emissions of gases that affect the creation of photochemical ozone in the lower atmosphere (smog) catalysed by sunlight.
Depletion of abiotic resources – minerals and metals	kg Sb-eq	Indicator of the depletion of natural non-fossil resources.
Depletion of abiotic resources – fossil fuels	MJ, net calorific value	Indicator of the depletion of natural fossil fuel resources.
Human toxicity – cancer, non-cancer	CTUh	Impact on humans of toxic substances emitted to the environment. Divided into non-cancer and cancer related toxic substances.
Eco-toxicity (freshwater)	CTUe	Impact on freshwater organisms of toxic substances emitted to the environment.
Water use	m3 world eq. deprived	Indicator of the relative amount of water used, based on regionalized water scarcity factors.
Land use	Dimensionless	Measure of the changes in soil quality (Biotic production, Erosion resistance, Mechanical filtration).
Ionising radiation, human health	kBq U-235	Damage to human health and ecosystems linked to the emissions of radionuclides.
Particulate matter emissions	Disease incidence	Indicator of the potential incidence of disease due to particulate matter emissions.

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Last phase: Interpretation: Finally, the last phase of an LCA is the interpretation phase. The results of the LCI and LCIA are discussed together to arrive to conclusions, recommendations and/or decision making according to the goal and scope defined in the first phase.

3.3.7.2 LCAs assessing AD and biogas production (LCA-AD)

First phase: goal and scope: The usual goal of an LCA-AD is to determine the environmental impact of the production and use of biogas as an energy source (Hijazi et al., 2016; Saracevic et al., 2019).

The functional unit of an LCA-AD depends on the choice of the authors of each study. Commonly, the functional unit which is used to estimate the impact of an AD activity is an amount of energy obtained from biogas. Alternatively, another functional unit that can be defined is an amount of feedstock processed by AD. In this way, possible functional units may be, for example, 1 kWh of electricity produced on a Combined Heat and power unit (CHP) with biogas as fuel; or 1 ton of processed fresh matter (Bacenetti et al., 2013). Other functional units may be TJ, MJ, m3 biogas, driving distance, etc (Hijazi et al., 2016).

Like the functional unit, the subsystems of an AD system differ among different LCA-ADs. Hijazi et al 2016 propose that the production of biogas using AD can be divided in four subsystems: feedstock supply, biogas production, digestate utilization and biogas utilization.

For the feedstock supply, the input of the subsystem is the feedstock prior to any treatment or transportation to the biogas plant, and the output is the substrate ready to feed the system of AD. The impact of this phase depends strongly in the type of feedstock. If it is an energy crop, all the steps involved in the cultivation of the crop must be assessed: land preparation, sowing, cultivation, harvest, and transport to the biogas plant. This considerably increase the impact of the use of biogas. Contrary, if the feedstock is composed of residues of the harvesting of a crop dedicated for food, the impact on the environment will decrease, as the feedstock can be considered as waste of another system. The same happens with animal slurry and industrial waste (Hijazi et al., 2016).

The biogas production subsystem is the AD process itself. The output of the previous phase is the input of this one (substrate). Microbial communities present in the feed sludge digest the substrate during the four phases that comprise the AD: hydrolysis, acidogenesis, acetogenesis and methanogenesis. The outputs of this phase are biogas and digestate.

Digestate-use subsystem only considers the impact produced by the storage of digestate, because the use of the digestate is outside of the system boundaries. It may be considered for example for the cropping that will use the digestate as fertilizer.

Regarding biogas-use subsystem, the impact measurement applies to produce a certain amount of energy and/or heat from biogas combustion. The way by which energy stored in CH_4 molecules is recovered will be very important for the impact measurement: if biogas is just combusted for heating or cooking, if it is used to feed a CHP unit, or if it is upgraded to biomethane to feed the gas grids (Morero et al., 2015).

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Second phase: LCI: For the inventory of the AD process, data can be obtained from several sources. It is very important that sources are reliable because the quality of an LCA strongly depends on the quality of the LCI (Bacenetti et al., 2013).

For example, Bacenetti et al 2013 performed an LCA comparing three biogas plants using different feedstocks: pig slurry, maize silage, or a combination of both. The authors performed surveys in farms and interviews to farmers to assess data on production and transport of maize; also surveys in biogas plants to assess data on pig slurry transport; they monitored biogas plants during a year for the data relative to biogas plant operation, such as biomass, electricity and heat consumed, amount of produced biogas, etc. They also supplemented this information with secondary data obtained from the Ecoinvent database (https://ecoinvent.org/), the biggest LCA database available. Finally, they used the IPCC method to assess the emissions derived from the use of fertilizers (https://www.ipcc.ch/2019/05/13/ipcc-2019-refinement/).

Third phase: LCIA: Environmental sustainability will be assessed based on the impact of a stage or the whole process, on several categories relevant to the AD of biomass, such as resource depletion, acidification, ozone depletion, global warming, human toxicity, eutrophication, and eco-toxicity.

Defining the functional unit and allocation are the major determinant components to assess the environmental sustainability of a biorefinery. This is due to its multi-functional nature, which causes ambiguity in the allocation of burdens to the other products of the system when only comparing the global warming impacts while ignoring other impacts (Katakojwala and Mohan, 2021).

In general, electricity from biogas has lower environmental impacts compared to electricity generation from fossil fuels. While savings in terms of GWP and RC can still be achieved, such biogas systems have higher impacts of AC, EP, and LU in comparison to fossil based energy systems (Hijazi et al., 2016).

Last phase: interpretation: The sustainability of any biorefining majorly depends on whether the feedstock is dedicated biomass or alternatively is a residue biomass/waste (Katakojwala and Mohan, 2021).

Regardless of the selection of the feedstock, the following steps are necessary to minimize the negative environmental effects of biogas systems (impact categories that are mostly tackled by each step are stated in brackets) (Hijazi et al., 2016):

- Installing a flare to avoid discharge of biogas to the atmosphere during outages of the combined heat-and-power unit (GWP);
- Covering the storage tank for digested residues and collecting the residual biogas production (GWP, EP);
- Minimizing the parasitic electricity demand of the biogas plant and supplying it from low-emission sources (GWP, RC);

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- Utilizing as much heat output from the CHPU as possible to substitute fossil energy carriers (GWP, RC);
- Employing high-efficiency CHPUs, possibly with additional exhaust gas treatment (RC, GWP, AC, EP);
- Checking the biogas plant for leakages on a regular basis (GWP)

References

- Bacenetti J, Negri M, Fiala M, González-García S. 2013. Anaerobic digestion of different feedstocks: Impact on energetic and environmental balances of biogas process. Sci. Total Environ. 463–464, 541–551.
- Bacenetti J, Sala C, Fusi A, Fiala M. 2016. Agricultural anaerobic digestion plants: What LCA studies pointed out and what can be done to make them more environmentally sustainable. Appl. Energy 179, 669–686.
- Baird C, Cann M. 2014. *Química ambiental.*
- Belgium. 2021 National Inventory Report (NIR) | UNFCCC: https://unfccc.int/documents/271642 (accessed 4.20.22).
- Belgium - Countries & Regions: https://www.iea.org/countries/belgium (accessed 4.24.22).
- BIOGAS3, European legislative and financial framework for the implementation of small-scale biogas plants in agro-food & beverage companies, 04-20-2016.
- Biogaz. Site Énerg. Serv. Public Wallonie: https://energie.wallonie.be/fr/biogaz.html?IDC=9235&IDD=96298 (accessed 4.20.22).
- Biokaasu2030. Suom. Biokierto Ja Biokaasu Ry: https://biokierto.fi/biokaasu/biokaasu2030/ (accessed 4.23.22).
- Biokaasu2030 Suom. Biokierto ja Biokaasu ry.
- BOE.es - Sumario del día 21/05/2021, www.boe.es/boe/dias/2021/05/21/ (accessed 4.14.22).
- EBA 2021. "Statistical Report of the European Biogas Association 2021." Brussels, Belgium, November 2021.
- Bourguignon D. 2018. Circular economy package. Comm. stafe Work. Doc., 74.
- Commission, E. (2020). Nuevo Plan de acción para la economía circular por una Europa más limpia y más competitiva. 2–32.
- Büsgen U, Dürrschmidt W. 2009. The expansion of electricity generation from renewable energies in Germany: A review based on the Renewable Energy Sources Act Progress Report 2007 and the new German feed-in legislation. Energy Policy. 37, 2536–2545.
- Christoph. » Federal Immission Control Act (Bundes-Immisionsschutzgesetz, BImSchG) – Excerpts German Law Archive: https://germanlawarchive.iuscomp.org/?p=315 (accessed 4.15.22).
- Del Rio P. 2008. Ten years of renewable electricity policies in Spain: An analysis of successive feed-in tariff reforms. Energy Policy. 36, 2917–2929.
- Energy Statistics Pocketbook. 2022. United Nations Department of Economic and Social Affairs.
- Erisman J W, Galloway J N, Seitzinger S, Bleeker A, Dise N B, Petrescu A M R, et al. 2013. Consequences of human modification of the global nitrogen cycle. Philos Trans R Soc Lond B Biol Sci. 368:20130116.
- FAO. 2021. *World Food and Agriculture - Statistical Yearbook 2021*. Rome Available at: https://doi.org/10.4060/cb4477en.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- European Commission. Directive 2018/851 of the European parliament and of the council amending directive 2008/98/EC on waste. Off J Eur Union. 2018:109–140.
- European Commission. Directive 2008/98/EC of the European parliament and of the council on waste and repealing certain directives. Off J Eur Union. 2008:3–30.
- European Commission. Communication from the commission to the European parliament, the council and the European economic and social committee of the Regions – A sustainable bioeconomy for Europe: strengthening the connection between economy, society and the environment Brussels, Belgium: 2018.
- European Commission. Directive 2018/2001 of the European parliament and of the council on the promotion of the use of energy from renewable sources (recast). Off J Eur Union. 2018: 82–206.
- European Commission. Directive 2009/28/EC of the European parliament and of the council on the promotion of the use of energy from renwable sources. Off J Eur Union. 2009:16–62.
- European Commission. Directive 1999/31/EC of the European parliament and of the council on the landfill of waste. Off J Eur Union. 1999:1–19.
- European Commission. Communication from the commission to the european parliament, the council and the european economic and social committee of the regions on an EU strategy to reduce methane emissions Brussels, Belgium: 2020.
- European Commission. Communication from the commission to the european parliament, the council and the european economic and social committee of the Regions - Powering a climate-neutral economy: an EU strategy for energy system integration Brussels, Belgium: 2020.
- Fagerström A, Al Seadi T, Rasi S, Briseid T, Murphy JD, IEA Bioenergy Task 37, IEA Bioenergy Programme, 2018. The role of anaerobic digestion and biogas in the circular economy.
- Finland. 2020 National Inventory Report (NIR) | UNFCCC: https://unfccc.int/documents/219060 (accessed 4.23.22).
- Finland - Countries & Regions, IEA: www.iea.org/countries/finland (accessed 4.20.22).
- Flórez G, Isabel M. 2017. Survey of Spanish biogas plants and their characteristics – the regions of Andalucía, Aragón, Asturias, Castilla la Mancha, Castilla y León, Galicia, Murcia and Navarra.
- Fowler D, Coyle M, Skiba U, Sutton MA, Cape J N, Reis S, et al. (2013). The global nitrogen cycle in the twenty- first century.
- Friedlingstein P, Sullivan MO, Jones MW, Andrew RM, Hauck J. 2020. Global Carbon Budget 2020. 2020, 3269–3340.
- Galloway JN, Aber JD, Erisman JANW, Sybil P, Howarth RW, Cowling EB, et al. 2003. The Nitrogen Cascade. 53, 341–356.
- Germany. 2021 National Inventory Report (NIR) | UNFCCC: https://unfccc.int/documents/273433 (accessed 4.23.22).
- Greece. 2021 National Inventory Report (NIR) | UNFCCC: https://unfccc.int/documents/272918 (accessed 4.20.22).
- Greece - Countries & Regions: https://www.iea.org/countries/greece (accessed 4.20.22).
- Gustafsson M, Anderberg S. 2022. Biogas policies and production development in Europe: a comparative analysis of eight countries. Biofuels 1–14.
- Hijazi O, Munro S, Zerhusen B, Effenberger M. 2016. Review of life cycle assessment for biogas production in Europe. Renew. Sustain. Energy Rev. 54, 1291–1300.
- Huttunen MJ, Kuittinen V, Lampinen A. 2018. Suomen biokaasulaitosrekisteri n:o 21: Tiedot vuodelta 2017. Itä-Suomen yliopisto.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Ingrao C, Bacenetti J, Adamczyk J, Ferrante V, Messineo A, Huisingh D. 2019. Investigating energy and environmental issues of agro-biogas derived energy systems: A comprehensive review of Life Cycle Assessments. Renew. Energy 136, 296–307.
- Katakojwala R, Mohan SV. 2021. A critical view on the environmental sustainability of biorefinery systems. Curr. Opin. Green Sustain. Chem. 27, 100392.
- Kwant KW. 2003. Renewable energy in The Netherlands: policy and instruments. Biomass Bioenergy, Proceedings of the IEA Bioenergy Task 31 Workshop “Principles and Practice of Forestry and Bioenergy in Densely-Populated Regions” 24, 265–267. https://doi.org/10.1016/S0961-9534(02)00175-7.
- Leach AM, Galloway JN, Bleeker A, Willem J, Kohn R, Kitzes J. 2012. A nitrogen footprint model to help consumers understand their role in nitrogen losses to the environment. Environ. Dev. 1, 40–66.
- Lovarelli D, Falcone G, Orsi L, Bacenetti J. 2019. Agricultural small anaerobic digestion plants: Combining economic and environmental assessment. Biomass and Bioenergy 128, 105302.
- Lyytimäki J, Nygrén NA, Pulkka A, Rantala S. 2018. Energy transition looming behind the headlines? Newspaper coverage of biogas production in Finland. Energy Sustain. Soc. 8, 15.
- Lyytimäki J, Nygrén NA, Pulkka A, Rantala S. 2018. Energy transition looming behind the headlines? Newspaper coverage of biogas production in Finland. Energy Sustain. Soc. 8, 15.
- Marchal D, Ryckmans Y, Crehay R, Schenkel Y. 2007. Green certificate mechanisms in Belgium: a useful instrument to mitigate GHG emissions, in: Proceedings of the 15th European Biomass Conference and Exhibition. pp. 3046–3051.
- Markou G, Brulé M, Balafoutis A, Kornaros M, Georgakakis D, Papadakis G. 2017. Biogas production from energy crops in northern Greece: economics of electricity generation associated with heat recovery in a greenhouse. Clean Technol. Environ. Policy 19, 1147–1167.
- Matschoss P, Bayer B, Thomas H, Marian A. 2019. The German incentive regulation and its practical impact on the grid integration of renewable energy systems. Renew. Energy 134, 727–738.
- Mayer F, Bhandari R, Gäth S. 2019. Critical review on life cycle assessment of conventional and innovative waste-to-energy technologies. Sci. Total Environ. 672, 708–721.
- Minelgaitė A, Liobikienė G. 2019. Waste problem in European Union and its influence on waste management behaviours. Sci. Total Environ. 667, 86–93.
- Ministerio de Industria, Energía y Turismo, 2014. Real Decreto 413/2014, de 6 de junio, por el que se regula la actividad de producción de energía eléctrica a partir de fuentes de energía renovables, cogeneración y resíduos.
- Netherlands. 2021 National Inventory Report (NIR) | UNFCCC: https://unfccc.int/documents/273459 (accessed 4.20.22).
- Morero B, Groppelli E, Campanella EA. 2015. Life cycle assessment of biomethane use in Argentina. Bioresour. Technol. 182, 208–216.
- Netherlands: energy production from biogas 2013-2018: https://www.statista.com/statistics/862760/biogas-energy-production-in-the-netherlands/ (accessed 4.8.22).
- Nevzorova T, Kutcherov V. Barriers to the wider implementation of biogas as a source of energy: a state-of-the-art review. Energy Strategy Rev. 2019;26:100414.
- New CO2 emissions tax in Germany | News and views: https://www.bp.com/en/global/air-bp/news-and-views/sterling-card-newsletters/new-co2-emissions-tax-in-germany.html (accessed 4.15.22).

This project has been granted funding from the European Union’s Horizon 2020 research and innovation programme

- Paes LAB, Bezerra BS, Deus RM, Jugend D, Battistelle RAG. 2019. Organic solid waste management in a circular economy perspective – A systematic review and SWOT analysis. J Clean Prod. 239:118086.
- Paolini V, Petracchini F, Segreto M, Tomassetti L, Naja N, Cecinato A. 2018. Environmental impact of biogas: A short review of current knowledge. J Environ Sci Heal. - Part A Toxic/Hazardous Subst Environ Eng. 53, 899–906.
- Poeschl M, Ward S, Owende P. 2010. Prospects for expanded utilization of biogas in Germany. Renew Sustain Energy Rev. 14, 1782–1797.
- Raboni M, Urbini G. 2014. Production and use of biogas in Europe : a survey of current status and perspectives Produção e utilização de biogás na Europa : um levantamento da situação atual e perspectivas. 9. doi:10.4136/1980-993X.
- Renewable energy use, 1990-2019 | Environmental Data Compendium: https://www.clo.nl/en/indicators/en0385-renewable-energy-use (accessed 4.15.22).
- Renewable energy targets: https://energy.ec.europa.eu/topics/renewable-energy/renewable-energy-directive-targets-and-rules/renewable-energy-targets_en (accessed 4.23.22).
- Romero-Rubio C, de Andrés Díaz JR. 2015. Sustainable energy communities: a study contrasting Spain and Germany. Energy Policy. 85, 397–409.
- Rutz WM. 2006. Dominik, n.d. BiG>East - Promoting Biogas in Eastern Europe: Mobilization Campaigns and Training: https://www.big-east.eu/greece/greece.html (accessed 4.9.22).
- Saracevic E, Koch D, Stuermer B, Mihalyi B, Miltner A, Friedl A. 2019. Economic and global warming potential assessment of flexible power generation with biogas plants. Sustain. 11:2530.
- Saunois M, Stavert AR, Poulter B, Bousquet P, Canadell JG, Jackson RB, et al. 2020. The Global Methane Budget 2000 – 2017. 1561–1623.
- Sharma B, Vaish B, Monika, Singh UK, Singh P, Singh RP. 2019. Recycling of Organic Wastes in Agriculture: An Environmental Perspective. Int. J. Environ. Res. 13, 409–429.
- Spain 2021 – Analysis, IEA: https://www.iea.org/reports/spain-2021 (accessed 4.20.22).
- Spain. 2020 National Inventory Report (NIR) | UNFCCC: https://unfccc.int/documents/228014 (accessed 4.20.22).
- Sawatdeenarunat C, Surendra KC, Takara D, Oechsner H, Khanal SK. 2015. Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. Bioresour. Technol. 178, 178–186.
- State aid: Commission approves Belgian certificates schemes for renewable electricity and high-efficiency cogeneration in Flanders. 2022. Eur. Comm.: https://ec.europa.eu/commission/presscorner/detail/en/IP_18_821 (accessed 4.8.22).
- Stegmann P, Londo M, Junginger M. 2020. The circular bioeconomy: Its elements and role in European bioeconomy clusters. Resour Conserv Recycl X. 6:100029. doi:10.1016/j.rcrx.2019.100029.
- Stein LY, Klotz MG. 2016. The nitrogen cycle. Curr Biol. 26, R94–R98.
- Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, et al. 2013. IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Available at: https://www.ipcc.ch/report/ar5/wg1/ (accessed 11.06.2022)
- Thomas H. 2019. Renewable Energy Sources Act 2017 in Germany—Auctions for Renewable Energy Transition. In: Palocz-Andresen M, Szalay D, Gosztom A, Sípos L, Taligás T (Eds.). International Climate Protection. Springer International Publishing, Cham, pp. 239–246. https://doi.org/10.1007/978-3-030-03816-8_30
- Timonen K, Sinkko T, Luostarinen S, Tampio E, Joensuu K. 2019. LCA of anaerobic digestion: Emission allocation for energy and digestate. J Clean Prod. 235, 1567–1579.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- US EIA (2013). International Energy Outlook 2013 - DOE/EIA-0484(2013). Outlook 2013, 312.
- Torrijos M. 2016. State of Development of Biogas Production in Europe. Procedia Environ. Sci., Waste Manag Resour Util 35. 881–889.
- Vertogas: https://www.vertogas.nl/ (accessed 4.14.22).
- Vanhamaki S, Medkova K, Malamakis A, Kontogianni S, Marisova E, Dellago DH, et al. (2019). Bio-based circular economy in European national and regional strategies. Int J Sustain Dev Plan. 14, 31–43.
- Vaverková MD. 2019. Landfill impacts on the environment— review. Geosci. 9, 1–16.
- Wainaina S, Awasthi MK, Sarsaiya S, Chen H, Singh E, Kumar A, et al. 2020. Resource recovery and circular economy from organic solid waste using aerobic and anaerobic digestion technologies. Bioresour. Technol. 301, 122778.
- Wang Z, Liu F. 2021. *Environmental assessment tools*. INC doi:10.1142/9789814354820_0021.
- Winquist E, Van Galen M, Zielonka S, Rikkonen P, Oudendag D, Zhou L, Greijdanus A. 2021. Expert Views on the Future Development of Biogas Business Branch in Germany, The Netherlands, and Finland until 2030. Sustainability 13, 1148.
- Zlokower R. 2019. World Biogas Association | Germany International Market report. World Biogas Assoc. URL https://www.worldbiogasassociation.org/germany-international-market-report/ (accessed 4.23.22).

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4. Policy of the European Union

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Abstract: This chapter addresses the main political instruments of the European Union that are intended to promote the growth of renewable energies. In particular, the promotion of biogas and bio-methane will be discussed in depth. This section aims to summarize the decisions, directives, and regulations of the EU and thus provide an ideal overview of the political starting position for biogas and bio-methane in the EU. First, the basis for the European climate policy and revolution, the European Green Deal, will be outlined. All further additions and procedures for the European Green deal will be presented chronologically in the following. In addition, sustainability provisions such as the Renewable Energy Directive (REDI & REDII), Paris Agreement, the gas embargo against Russia, the promotion of the bio-economy, the use of bio-waste are put into context for the production of biogas and bio-methane.

In the following, all the important decisions, directives, and regulations and their significance for the biogas sector are presented. For this, however, it must first be clarified what decisions, directives, recommendations, opinions, and regulations are and what they mean for the individual countries.

4.1 Regulations of the European Union

This chapter compares the biogas and bio-methane sectors of European countries. The 27 EU countries are discussed, as well as the United Kingdom, Norway, Switzerland, Ukraine, and Iceland as important biogas and bio-methane producers. In 2020, 191 TWh of biogas and bio-methane were produced in the entire European Union. This corresponds to about 4.6% of gas consumption in the EU. **Figure 60** shows the development of combined biogas and bio-methane production from 2011 to 2020.

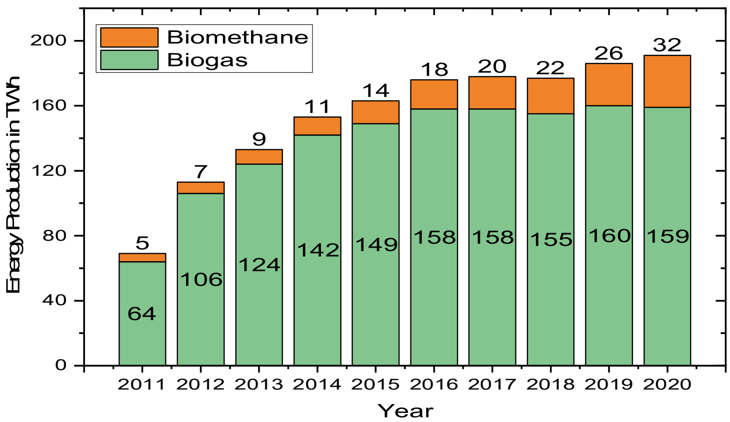

Figure 60: Trends and developments in biogas and bio-methane production in Europe. (European Biogas Association 2021)

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While the development of biogas production is stagnating because it is difficult to generate usable electricity and heat. Particularly the interest in the production of bio-methane has risen sharply in the last ten years. In contrast, bio-methane production is growing strongly due to high demand, storage capacity, and rising prices for natural gas. In 2019, 26 TWh of bio-methane were produced, and in the following year already 32 TWh, which means an increase of 23 %. A total of 161 new methane plants came on stream during this period. The United Kingdom saw the strongest bio-methane growth in 2020, with an increase of 1,689 GWh. This is followed by Denmark, with an increase of 1,347 GWh, France, with an increase of 972 GWh, Italy, with an increase of 805 GWh and the Netherlands, with an increase of 698 GWh. In terms of absolute numbers, Germany leads as a methane producer with 11,200 GWh, followed by the United Kingdom, with a production of 6,944 GWh, and Denmark, with a production of 4,041 GWh. The amount of biogas and bio-methane production varies greatly between European countries due to the respective national governments. Germany, for example, has many biogas plants due to the introduction of the Renewable Energy Sources Act and the feed-in tariff, while countries like Sweden have more bio-methane plants due to the promotion of bio-methane as a fuel. In general, however, the statistics show that the production of bio-methane is on an upward trend.

Figure 61 shows the countries that produce the most biogas and bio-methane. Germany produces the most biogas and bio-methane with 82.428 GWh. As mentioned above, this is due to the strong promotion of biogas production with the EEG of the German government. Germany is followed by the United Kingdom and Italy, which produce about one-third of what Germany produces.

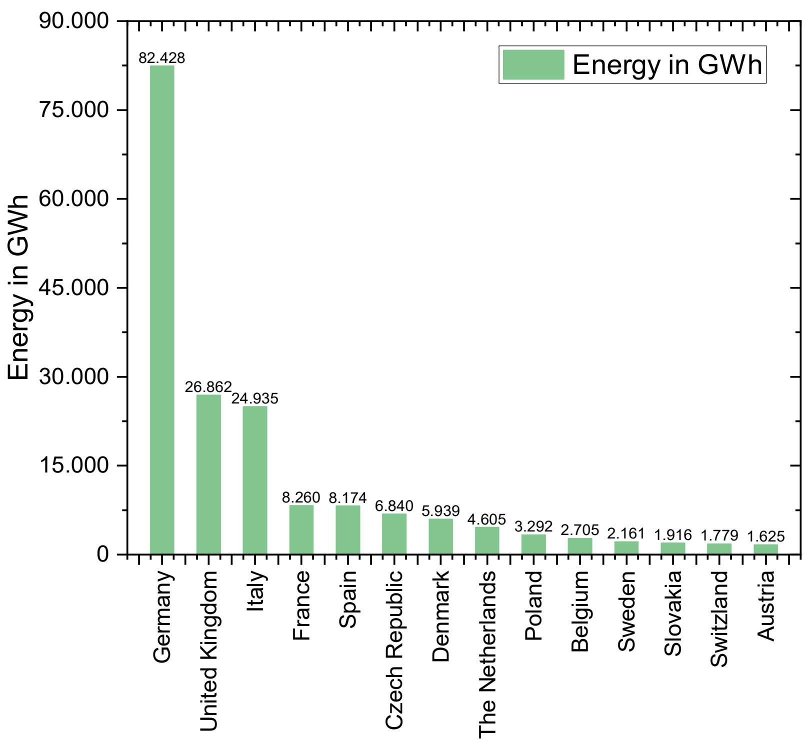

Figure 61: Top Biogas producers in Europe given in GWh. (European Biogas Association 2021)

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The largest share of biogas plants, 63%, is found in the agricultural sector. In comparison, landfills account for the second-largest share, 15 %. Among the bio-methane plants, the largest share is also found in the agricultural sector with a share of 53 %, whereas organic municipal waste is the second-largest source of bio-methane production with 11 %. (European Biogas Association 2021)

4.2 Regulations of the European Union

In European law, regulations are legal acts of the European Union and as such form part of secondary Union law. According to Art. 288 (2) of the TFEU, they are of general application and have direct effect in the Member States. Regulations are addressed either to the European Union itself, to all Member States or to the citizens of all Member States. the regulation is to affect only selected member states or their citizens, it is issued as a decision, which means it is directly binding, or as a directive, which must be transposed into national law. Regulations are adopted on the basis of one of the procedures provided for in the Treaties, depending on the subject of the regulation. A distinction is made between legislative acts, Commission implementing regulations and delegated regulations.

Directives, which are legislative acts, are usually adopted jointly by the Council of the European Union and the European Parliament in accordance with the ordinary legislative procedure, on a proposal from the European Commission. They are published in the Official Journal of the European Union and are available online in the legal information system EUR-Lex. Regulations are numbered with the word "Regulation", the year, a serial number and the symbol "EU".

In summary, regulations set the goals and the concrete means to achieve the results. They do not have to be transposed into national law, which is referred to as the "pass-through effect", and modifications are protected by the "transposition prohibition".

4.3 Directives of the European Union

In European law, directives are legal acts of the European Union and as such part of secondary Union law. According to Art. 288(3) of the TFEU, they are binding and not directly applicable, which means that they must first be converted into national law by the Member States. The form in which they are then implemented by the individual member states depends on them, which leaves them a certain amount of leeway. However, there are authorizations and/or obligations that must be complied with and which provide the framework for the directives.

Directives, which are legislative acts, are usually adopted jointly by the Council of the European Union and the European Parliament in accordance with the ordinary legislative procedure, on a proposal from the European Commission. They are published in the Official Journal of the European Union and are available online in the legal information system EUR-Lex. Directives are numbered with the word "Directive", the year, a serial number, and the symbol "EU".

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In summary, directives set objectives that must be achieved without specifying the means to achieve that result.

4.4 Decisions of the European Union

In European law, decisions are legal acts of the European Union and as such form part of secondary Union law. Alongside regulations and directives, decisions are the third form of EU legislative instrument that have legally binding effects on each individual. Depending on the subject, decisions are adopted through different, treaty-bound procedures. According to Art. 288 TFEU, decisions are binding in their entirety and may be addressed to specific addressees such as Member States, companies, individuals or the general public. Decisions are used when a resolution is to be binding but there is no case for the adoption of regulations or directives. This is the case in the following examples:

- Case-by-case decisions
- Nominations
- Common Foreign and Security Policy
- International Agreements
- Simplified Amendments to the Treaties

In this way, decisions are used to react to individual problems and abuses, such as mergers of companies, actions that endanger security, non-compliance with EU directives and regulations, the introduction of hazardous substances and the use of genetic engineering.

4.5 Further political agreements

The Treaty on European Union states that "In order to ensure the proper functioning and development of the common market, the Commission (...) formulate recommendations or deliver opinions on matters dealt with in this Treaty, if it expressively so provides or if the Commission considers it necessary." (European Union 1993)

Recommendations are part of the secondary legislation of the European Union and thus legal acts of the Union. Recommendations are usually issued by the European Commission. They are defined in Art. 288 TFEU as non-legally binding acts, although some legal consequences are attached to their adoption. Member States are free to decide whether to implement the recommendations. If they implement a recommendation, it is part of the legal order of the Member State, which means that the resulting rights can only be enforced before the courts of the Member State, not before the European Court of Justice. This makes the recommendation to an instrument of indirect action. Similar to the directive, it aims at the development of legislation in the member states, whereby recommendations are not binding.

In addition, the institutions of the European Union adopt other legally non-binding acts, such as resolutions, declarations, or conclusions, sometimes without an explicit legal basis.

Opinions are part of the secondary legislation of the European Union and thus legal acts of the Union. Opinions are issued by the institutions and other bodies of the Union. They are defined in Art. 288 TFEU as non-legally binding acts, although legal consequences are attached to their delivery. Two important areas in which the submission of opinions is provided

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for are mentioned below. Procedures for the adoption of legislative acts and infringement procedures.

4.6 European Green Deal

The European Green Deal is to become a central component of the European Union's climate policy and addresses the anthropological climate crisis in its core issues. This was adopted on 11 December 2019 by the European Commission under the leadership of Ursula von der Leyen to reduce net greenhouse gas emissions in the European Union to zero by 2050 at the latest. This is expected to make Europe the first continent to achieve climate neutrality. The European Green deal is a growth strategy to make the EU a prosperous and fair society with a competitive, modern, and resource-efficient economy, decoupling economic growth from unsustainable resource use (The European Commission 2019). It identifies action strategies for the sectors with the highest greenhouse gas (GHG) emissions, including the transport sector, construction sector, agriculture, forestry, industry, and general energy production. In addition, promising systems such as the circular economy, an emissions trading system at the EU and global level, and tax relief for the sustainable use of resources are discussed. A European Union procedural plan for the implementation of the Green Deal is shown in **Figure 62**.

Figure 62: The European Green Deal (The European Commission 2019)

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To create a holistic transformation, the Green Deal policy focuses on clean and fair energy supply to the entire economy and society, including the sectors of:

- Industry
- Production and consumption
- Large-scale infrastructure
- Transport
- Food and agriculture
- Construction
- Taxation and social benefits

This opens-up new opportunities for innovation, investment, and jobs, especially in connection with:

- Reduction of emissions
- Creation of growth and jobs in the sustainable sector
- Reduction of energy poverty
- Reducing energy dependence on third countries
- Improving our health and living conditions

All areas of action are closely interconnected and can reinforce each other in worst-case and best-case scenarios. Therefore, attention must be paid to possible conflicts between economic, environmental, and social goals. To create social justice transformation, the EU uses every political tool at its disposal:

- Regulation and standardisation
- Investment and innovation
- national reforms
- dialogue with social partners
- International cooperation

In the Green Deal, some goals and decisions are formulated relatively vaguely, as they will be defined more clearly in the course of the Green Deal. To understand the references and effects of the Green Deal holistically, all other important decisions and agreements especially related to biogas and bio-methane are presented chronologically below.

4.7 Decisions, directives, and regulations addressing the biomass sector

In the following, current EU-wide decisions, regulations, and directives are briefly presented. These landmark decisions are presented chronologically in the form of a timeline. In addition to information on the respective decision, the decision year, and the framework, the substantive objectives are presented in a compact summary. Furthermore, the specific reference to biogas and bio-methane is drawn and the effects of these resolutions on the biogas sector are presented.

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4.7.1 Directive 2008/98/EC: The Waste Framework Directive (WFD) // November 2008

The WPD, as a European Community Directive, provides the member states with guidelines for political measures for the transition to a circular economy and for their waste legislation. The aim is to protect the environment and human health by preventing or reducing the damaging effects of the generation and management of waste, reducing the overall impact of resource use, and improving the efficiency of resource use, thus ensuring the long-term competitiveness of the EU. (The European Parliament and Council of the European Union 2008)

4.7.2 Directive 2009/28/EC: Renewable Energy Directive I (REDI) // June 2009

RED I includes the expansion of renewable energies in the EU. By 2020, the EU member states committed themselves to a share of renewable energies in total energy consumption of at least 20 %. The feed-in of electricity generated with renewable energies is promoted via two funding systems. The feed-in model through the Renewable Energy Sources Act, where the producer is guaranteed a fixed feed-in tariff, and the quota model, where the state sets a fixed quota of renewable energy and the energy producer guarantees this using a certificate trading system. (The European Parliament and of the Council 2009)

4.7.3 Sustainable Development Goals (SDGs) // September 2015

The SDGs (**Figure 63**) were adopted by 193 parties in the United Nations General Assembly in 2015 and are to be achieved by 2030. The SDGs comprise a set of 17 interlinked global goals. These include (1) No Poverty, (2) Zero Hunger, (3) Good Health and Well-being, (4) Quality Education, (5) Gender Equality, (6) Clean Water and Sanitation, (7) Affordable and Clean Energy, (8) Decent Work and Economic Growth, (9) Industry, Innovation and Infrastructure, (10) Reduced Inequality, (11) Sustainable Cities and Communities, (12) Responsible Consumption and Production, (13) Climate Action, (14) Life Below Water, (15) Life On Land, (16) Peace, Justice, and Strong Institutions, (17) Partnerships for the Goals. These goals are intended to pave the way for a future characterized by peace, sustainability, a healthy environment, a stable climate, and environmentally aware people with socio-ecological prosperity. (United Nations 2015)

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Figure 63: Sustainable Development Goals (United Nations 2015)

In the further versions, the 17 goals were visualized and reformulated for areas that were particularly affected. (United Nations 2018)

4.7.4 Paris Agreement / December 2015

International treaty to counter the climate crisis, comprehensively addressing climate change mitigation, adaptation, and finance. The agreement includes limiting the effects of anthropogenic global warming to 2 °C and was ratified by 192 parties at the United Nations Climate Change Conference in 2015. The Paris Climate Agreement, together with the Kyoto Protocol and the Montreal Protocol, are among the most important environmental agreements in the history of the world. (United Nations Framework Convention on Climate Change 2015)

4.7.5 Closing the loop - An EU action plan for the Circular Economy // December 2015

In December 2015, the European Commission published the Circular Economy Package, initiating one of the most significant changes in environmental and economic policy in recent years. The aim is to create a more circular economy, which is about preserving the value of products, materials, and resources within the economy for as long as possible and generating as little waste as possible. Intelligent product design, more recycling, and reuse should help to increasingly close the loop of product life cycles and achieve more effective value creation and use of all raw materials, products, and waste. (The European Commission 2015)

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4.7.6 A sustainable bio-economy for Europe // November 2018

The Action Plan "A sustainable bio-economy for Europe" was published in November 2018 as a roadmap for the future of agriculture, economy, and industry. Bio-economy refers to the economic use of renewable biological resources from land and sea, such as plants, forests, fish, animals, and microorganisms for the production of food, materials and energy. This strategy aims to help the EU reduce carbon emissions and achieve a circular economy. The bio-economy will contribute to and strengthen the modernization of the EU's industrial sector by establishing new value chains and greener, more cost-efficient industrial processes while protecting biodiversity and the environment. (European Commission 2018)

4.7.7 Directive (EU) 2018/2001: Renewable Energy Directive II (REDII) // December 2018

RED II entered into force on 24 December 2018 and was transposed into national legislation on 30 June 2021, which meant that RED I expired. In contrast to RED I, the EU Commission has set a binding target of 32% renewable energy for the total electricity production of all EU member states by 2030. They are committed to achieving the 32% through the National Energy and Climate Plans (NECP). The achievement of this overall target is regulated for each member state in Art. 3 Para 1-9 of the Governance Regulation. (The European Parliament and Council of the European Union 2018b) The states must submit measures, timetables, and proposals for financing to the EU Commission, which may be adjusted by the EU Commission if necessary. (The European Parliament and Council of the European Union 2018a) The commitment of 32% renewable energy for the electricity sector by the National Energy and Climate Plans (NECP) was adjusted to 40% under the 'fit-for-55' program.

4.7.8 European Green Deal // December 2019

The European Commission presents the European Green Deal to achieve climate neutrality by 2050. The European Green Deal comprises a series of measures in the areas of financial market regulation, energy supply, transport, trade, industry and agriculture, and forestry. Initially, the European Union's CO_2 emissions were to be reduced by 40 percent by 2030 compared to 1990 levels. Subsequently, the reduction was first tightened to 50 and finally to 55 percent. Particularly affected countries will be supported with a total of 100 billion euros in the conversion to an emission-free economy. (The European Commission 2019)

4.7.9 Circular Economy Action Plan – For a cleaner and more competitive Europe // March 2020

4.7.10 Regulation (EU) 2020/852: EU taxonomy for sustainable activities // June 2020

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The EU Taxonomy for Sustainable Activities is a classification system that aims to clarify which investments are environmentally sustainable, building on the European Green Deal. The classification system aims to prevent greenwashing and help investors make greener choices. For this purpose, investments are assessed against six objectives: Climate Change Mitigation, Climate Change Adaptation, Circular Economy, Pollution, Water Impact, and Biodiversity. (The European Parliament and Council of the European Union 2020)

4.7.11 2020/2077(INI) – New Circular Economy Action Plan // February 2021

On 10 February, the European Parliament's presented the new circular economy action plan and in March 2020 the action plan was adopted by European Commission. The aim is for Europe to grow into a sustainable economy, which will make it possible to achieve the targets of the European Green Deal. This should reduce the pressure on natural resources, create new jobs and think more holistically about the cycle of life. In addition, the entire life cycle of products is to be considered in the future, whereby a central component of product design will be circularity. In addition to these economic instruments, sustainable consumption by the population is to be encouraged. Overall, these measures will ensure that the resources used are utilised in the EU economy for as long as possible, resulting in an optimal and justified appreciation of the resources. Therefore, the Action Plan is an important building block in achieving the EU's goal of climate neutrality by 2050 and halting the loss of biodiversity.

4.7.12 Fit-for-55-Paket // July 2021

On 14 July 2021, the "Fit for 55 Package" was published It contains more than ten cross-cutting legislative proposals that reshape existing climate, energy, and transport legislation. Commission presents a package of proposals to transform our economy to meet our 2030 climate targets. Negotiation and adoption of the legislative package by the European Parliament and the Member States to achieve our 2030 climate targets. The aim is to reduce net greenhouse gas emissions by 55% by 2030 compared to 1990 levels. The focus is on the following objectives: the breakdown of the 55% target, the reform of the European Emissions Trading Scheme, the introduction of a CO2 limit compensation system, the adaptation of the EU Climate Protection Regulation, the adaptation of the Renewable Energy Directive and the Climate, Environment, and Energy Aid Guidelines. By the end of 2022, all EU member states must develop strategic plans that will guide the allocation of funds. Furthermore, a climate fund of 72.2 billion euros has been set up as part of the Fit-for-55-Paket. This is intended for the period from 2025 to 2032. (The European Commission 2021)

4.7.13 REPowerEU

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4.8 EU policy related to Biogas and Bio-methane

In this chapter, explicit conclusions are drawn in which areas the respective package addresses the production and use of biogas and bio-methane. In this context, the policy instruments mentioned in previous sub-chapters will be discussed.

The Waste Framework Directive (Directive 2008/98/EC): The Waste Framework Directive primarily describes the collection, sorting, and treatment of waste and contains some directives for this purpose.

(1) "It is important, in accordance with the waste hierarchy, and for the purpose of reduction of greenhouse gas emissions originating from waste disposal on landfills, to facilitate the separate collection and proper treatment of bio-waste in order to produce environmentally safe compost and other bio-waste based materials." (The European Parliament and Council of the European Union 2008, L 312/7)

This directive covers the production of biogas at 2 points. Firstly, separately collected bio-waste is a valuable secondary resource that can be used in pure form as a substrate for the production of biogas and bio-methane. Secondly, this process prevents the release of methane, which is harmful to the climate. Instead, it is collected and used as fuel. Additionally, the remaining fermentation residue can be ideally used as fertiliser.

(2) "animal by-products including processed products covered by Regulation (EC) No 1774/2002, except those which are destined for incineration, landfilling or use in a biogas or composting plant;" (The European Parliament and Council of the European Union 2008, L312/9)

The handling of animal excreta is not covered in the WFP. Instead, reference is made to Regulation (EC) No 1774/2002, which specifically describes the requirements for a biogas plant using animal excrement as substrate. (The European Parliament and of the Council 2002)

(3) "'bio-waste' means biodegradable garden and park waste, food and kitchen waste from households, restaurants, caterers and retail premises and comparable waste from food processing plants;" (The European Parliament and Council of the European Union 2008, L 312/9)

The collection of these secondary raw materials provides a good substrate basis for biogas plants in highly populated areas.

(4) "Member States shall take measures, as appropriate, and in accordance with Articles 4 and 13, to encourage: (a) the separate collection of bio-waste with a view to the composting and digestion of bio-waste; (b) the treatment of bio-waste in a way that fulfils a high level of environmental protection; (c) the use of environmentally safe materials produced from bio-waste. The Commission shall carry out an assessment on the management of bio-waste with a view to submitting a proposal if appropriate. The assessment shall examine the opportunity of setting minimum requirements for bio-waste management and quality criteria for compost and digestate from bio-waste, in order to guarantee a high level of protection for human health and

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the environment." (The European Parliament and Council of the European Union 2008, L 312/16)

This guideline also addresses the optimal use of bio-waste as a substrate for preferably biogas plants.

(5) "Waste management plans shall conform to the waste planning requirements laid down in Article 14 of Directive 94/62/EC and the strategy for the implementation of the reduction of biodegradable waste going to landfills, referred to in Article 5 of Directive 1999/31/EC." (The European Parliament and Council of the European Union 2008, L312/7)

In future, bio-waste is to be better collected and separated in the EU countries. This material feed can underline the economic viability of biogas plants, especially in regions with a high population density. With these short transport routes, the waste heat, the electricity and the biomethane can be used effectively.

<u>Points of criticism:</u>

- Poor control of the implementation of waste collection especially the separate collection of bio-waste
- Penalties too low for illegal waste disposal, environmental pollution, and incorrect separation
- More education and seminars for correct separation especially for young adolescents
- Concepts and promotion for the collection companies dedicated to bio-waste

Renewable Energy Directive I: The Renewable Energy Directive serves primarily as a foundation for the European Renewable Energy Policy. It includes binding targets at EU level, National Climate Change Plans (NECP), support schemes, prohibition of deterioration, heating-cooling sector, transport sector. This directive was updated in 2018 by RED II. Despite this, RED I is a good basis to discuss the main points.

(1) "The use of agricultural material such as manure, slurry, and other animal and organic waste for biogas production has, in view of the high greenhouse gas emission saving potential, significant environmental advantages in terms of heat and power production and its use as biofuel. Biogas installations can, as a result of their decentralised nature and the regional investment structure, contribute significantly to sustainable development in rural areas and offer farmers new income opportunities." (The European Parliament and of the Council 2009, L 140/17)

To comply with the renewable energy directive, agricultural residues such as animal excreta and organic waste are to be used to produce biogas. On the one hand, the emission of methane is reduced, as the waste is not just deposited in the environment, and on the other hand, valuable resources such as heat, electricity, and biofuels are obtained.

(2) "'energy from renewable sources' means energy from renewable non-fossil sources, namely wind, solar, aerothermal, geothermal, hydrothermal and ocean energy, hydropower, biomass, landfill gas, sewage treatment plant gas and biogases;" (The European Parliament and of the Council 2009, L 140/27)

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This phrase ensures that biogas is classified as a regenerative energy source.

(3) "When designing their support systems, Member States may encourage the use of biofuels which give additional benefits, including the benefits of diversification offered by biofuels made from waste, residues, non-food cellulosic material, ligno-cellulosic material and algae, as well as nonirrigated plants grown in arid areas to fight desertification, by taking due account of the different costs of producing energy from traditional biofuels on the one hand and of those biofuels that give additional benefits on the other. Member States may encourage investment in research and development in relation to those and other renewable energy technologies that need time to become competitive." (The European Parliament and of the Council 2009, L 140/26)

The production of biogas and especially the purification into bio-methane also specifically addresses the production of bio-fuels with the help of processed waste. The efficiency of the fermentation of materials such as lignin, cellulose, and other plant raw materials is increased with progressive research.

In the following, the greenhouse saving potentials through the production of biogas from animal excreta and organic waste are listed. With 23 times the greenhouse gas potential, methane is even more harmful to the climate than carbon dioxide. By using resources such as animal excrement and organic waste, the methane produced can be captured and made usable. Thus, the construction of biogas plants with the use of waste leads to the reduction of climate-damaging gases and the development of an intact circular economy and bioeconomy.

Points of criticism:

- Biogas plants should also be built in urban regions, as the heat, in particular, can be used more effectively there
- Biofuels have some disadvantages that should also be critically examined (fine dust production, general combustion reaction)
- Potentials of biogas plants should be ideally exploited (effective use of heat, methane, electricity, fertilizer)
- Emphasising the relevance for industry, as biogas plants produce continuously and do not depend on wind or sun

Sustainable Development Goals: The sustainable development goals are a global non-profit project that was initiated by the United Nations in 2015. The aim is to make the earth a better place with 17 goals, where people, animals, living beings, and the environment can coexist peacefully.

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4.9 Current crisis influencing the policy for the biogas sector

European dependence on gas imports from Russia combined with Russia's war against Ukraine led to an energy price crisis. This war is prompting a rethink of Europe's resource and energy policy, especially with politically unstable regions and states with totalitarian structures. For this purpose, the EU Commission presented a communication on remedial measures on 8 March 2022. These include accelerating the expansion of renewable energies by speeding up planning and approval procedures. Originally, in the Green Deal, the European Commission had set the target for biomethane or biogas production at 17 billion m^3 by 2030. For the occasion, the target was raised to 35 billion m^3. From today's level, this means a tenfold increase in current production in the EU. (The European Commission 2022) In detail, independence from Russian fossil fuels will be achieved by 2030 as follows:

- Diversifying gas supplies
- Larger volumes of biomethane and renewable hydrogen production and imports
- Reducing faster the use of fossil fuels in our homes, buildings, industry, and power system
- Boosting energy efficiency
- Increasing renewables and electrification
- Addressing infrastructure bottlenecks

References

- European Biogas Association (2021): Statistical Report of the European Biogas Association 2021. Brussels, Belgium. Brussels, Belgium.
- European Commission (2018): A sustainable bioeconomy for Europe. Strengthening the connection between economy, society and the environment: updated bioeconomy strategy. Luxembourg: Publications Office of the European Union.
- European Union (1993): Treaty on European Union. Maastricht. In: Official Journal of the European Communities.
- The European Commission (2015): Closing the loop - An EU action plan for the Circular Economy. Brussels, Belgium. In: Official Journal of the European Union.
- The European Commission (2019): The European Green Deal. Brussels, Belgium. In: Official Journal of the European Union.
- The European Commission (2021): 'Fit for 55': delivering the EU's 2030 Climate Target on the way to climate neutrality. Brussels, Belgium 2021.
- The European Commission (2022): REPowerEU: Joint European action for more affordable, secure and sustainable energy. Strasbourg, France.
- The European Parliament and Council of the European Union (2008): Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 on waste and repealing certain Directives. Brussels, Belgium. In: Official Journal of the European Union 2008.
- The European Parliament and Council of the European Union (2018a): Directive (EU) 2018/2001 of the European Parliament and of the Council of 11 December 2018 on the promotion of the use of energy from renewable sources (recast): RED II. Brussels, Belgium. Brussels, Belgium. In: Official Journal of the European Union 2018.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- The European Parliament and Council of the European Union (2018b): Regulation (EU) 2018/1999 of the European Parliament and of the Council of 11 December 2018 on the Governance of the Energy Union and Climate Action, amending Regulations (EC) No 663/2009 and (EC) No 715/2009 of the European Parliament and of the Council, Directives 94/22/EC, 98/70/EC, 2009/31/EC, 2009/73/EC, 2010/31/EU, 2012/27/EU and 2013/30/EU of the European Parliament and of the Council, Council Directives 2009/119/EC and (EU) 2015/652 and repealing Regulation (EU) No 525/2013 of the European Parliament and of the Council. Brussels, Belgium. In: Official Journal of the European Union 2018.
- The European Parliament and Council of the European Union (2020): Regulation (EU) 2020/852 of the European Parliament and of the Council of 18 June 2020 on the establishment of a framework to facilitate sustainable investment, and amending Regulation (EU) 2019/2088. Brussels, Belgium. In: Official Journal of the European Union 2020.
- The European Parliament and of the Council (2002): Regulation (EC) No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down health rules concerning animal by-products not intended for human consumption. Brussels, Belgium. In: Official Journal of the European Union.
- The European Parliament and of the Council (2009): Directive 2009/28/Ec of the European Parliament and of the Council of 23 April 2009 on the Promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. Brussels, Belgium. In: Official Journal of the European Union. Online verfügbar unter http://www.nezeh.eu/assets/media/fckuploads/file/legislation/red_23april2009.pdf.
- United Nations (2015): Transforming our world: the 2030 agenda for sustainable development. New York, NY, USA.
- United Nations (2018): The 2030 Agenda and the Sustainable Development Goals: An opportunity for Latin America and the Caribbean. Santiago. Online verfügbar unter https://repositorio.cepal.org/handle/11362/40156.4.
- United Nations Framework Convention on Climate Change (2015): Paris Agreement. Paris, France 2015.

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5. Microorganisms involved in anaerobic digestion

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5.1 Archaea

Archaea are classified as the third domain of life along with prokaryotes and eukaryotes. They possess unique characteristics that distinguish them from bacterial prokaryotes. These features include novel membrane lipids based upon phytanyl ether lipids and information processing systems like those found in eukaryotes (Liu et al., 2012). They were initially found in extreme environments with extreme conditions such as high salinity and high temperature, and environments in which they evolved to metabolize organics such as methane and nitrogen (Cabello et al., 2004; Singh et al., 2012). In addition to inhabiting extreme circumstances, archaea are widely distributed from terrestrial to aquatic environments.

Members of Crenarchaeota and Euryarchaeota are globally distributed, and some lineages, often uncultivated ones, are abundant in waters, soils and sediments (Bomberg et al., 2008; Timonen and Bomberg, 2009; Schleper and Nicol, 2010). For Euryarchaeota, the role those ubiquitous methanogens carry out in the environment is well characterized. Formation of methane in the final degradation step of organic matter under anaerobic conditions is an exclusive attribute of archaea. However, some Euryarchaeota participate in syntrophic anaerobic methane oxidation and are called as anaerobic methane oxidizers (Orphan et al., 2001). In relation to the nitrogen cycle, some cultured archaea (both Euryarchaeota and Crenarchaeota) are capable of denitrification and nitrogen fixation. Furthermore, ammonia-oxidizing archaea contribute greatly to the ammonia oxidation process. This is an important step of the nitrification pathway responsible for nitrogen leaching and N2O emissions and thought to impede plant nitrogen use efficiency in agricultural systems. In addition, studies have suggested that some Euryarchaeota may also degrade other hydrocarbons (Laso-Pérez et al., 2016; Dombrowski et al., 2017). Apart from these functions, they could be still involved in other biogeochemical processes such as sulfur and iron metabolism (Ofre et al., 2013). For example, several archaea utilize sulfur compounds as electron donors or acceptors for energy production (Kletzin, 2007). For iron, Fe(III) serves as terminal electron acceptor for anaerobic respiration by a variety of archaea, while Fe(II) serves as electron donor and/or energy sources for archaeal growth (Dong et al., 2021).

Since the discovery of the Archaea as a distinct domain in the late 1960s, scientists have come to recognize that Archaea represent a major proportion of microbial biomass on the planet. The increase in knowledge over archaea has expanded our view of the tree of life and early archaeal evolution, and has provided new insights into archaeal cultivation, functions and manipulation.

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5.1.1 Methanogenic archaea

Methanogenic archaea are a highly catabolically specialized group, and the only group responsible for CH_4 production, a very potent greenhouse gas. This gas is produced under anoxic conditions in numerous natural environments such as oceans, estuaries, soils, and lakes (Wen et al., 2017). Its emission from natural environments such as wetlands, oceans, and sediments accounts for over 70% of atmospheric methane globally (IPCC, 2007). However, they could be found in multicellular organisms (i.e., rumen fluid and digestive tracts) (Saengkerdsub and Ricke, 2013). In another situation, methanogenesis could be encountered in geothermal environments where gases (carbon dioxide and hydrogen gas) are the substrates used by methanogens.

Despite their broad phylogenetic diversity and distribution in different habitats, methanogens metabolize a very narrow range of substrates. A major consequence of this specialization is that methanogens are dependent microorganisms to provide electron donors and are typically outcompeted for those electron donors by more efficient metabolic processes such as nitrate, iron, and sulfate reduction. The substrates can be converted by syntrophic interaction of methanogens and fermenting bacteria that closely cooperate in methanogenic degradation of fatty acids, alcohols, most aromatic compounds, and amino acids (Worm et al. 2010).

The biological formation of methane is performed in three catabolic pathways (Figure 1): hydrogenotrophic, growth on H_2 + CO_2 with CO_2 serving as both carbon source and electron acceptor; methylotrophic, the reduction of the methyl group of methyl-containing compounds to methane and acetotrophic, the cleavage of acetate to CH_4 + CO_2. Central to all those pathways is the Wood–Ljundahl pathway and the most widely used substrate for methanogenesis is acetate, which accounts for two-thirds of the methane generated in environments. However, the hydrogenotrophic pathway comprises the largest phylogenetic diversity found in methanogens. During growth on H_2 + CO_2, CO_2 is first bound to methanofuran and thereby reduced to a formyl group. The formyl group is transferred to tetrahydromethanopterin (H4MPT) and reduced to a methyl group through the costs transferring 1.7 Na+/CH_4 over the membrane (Schlegel and Muller, 2011). Then methyl-CoM undergoes by the thiolate anion of HS-CoB thus liberating CH_4. In the methylotrophic pathway, small methylated carbon compounds like methanol and methylated amines are used (Kurth et al., 2020). Three quarters of the methyl groups are reduced to methane whereas one quarter is oxidized to carbon dioxide (Kurth et al., 2020). Methanogenesis from acetate starts with the activation of acetate to acetyl-CoA, followed by an oxidation of acetyl-CoA to CO_2 and a coenzyme-bound methyl group that is reduced to CH_4 with electrons gained during the oxidation reaction (Schlegel and Muller, 2011).

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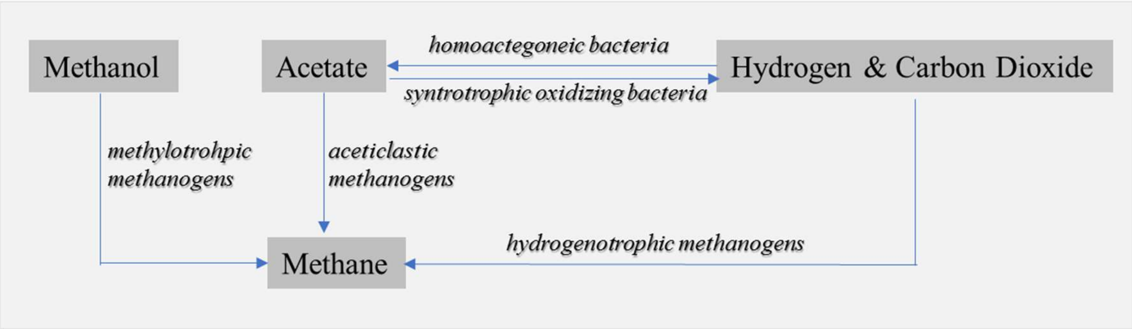

Figure 64: Overview of the main pathways leading to methane production

Our knowledge of methanogenic substrate utilization and energy conservation is still incomplete but is rapidly expanding through the development of new molecular techniques such as metagenomics. This includes the discovery of hydrogenotrophic and acectilactic methanogenesis genes encoded for different taxa.

5.1.2 Biology of methanogenic archaea

Taxonomically, all methanogens are members of the kingdom Euryarchaeota, but they form a broad group comprising 7 orders (Methanobacteriales, Methanococcales, Methanomicrobiales, Methanopyrales, Methanomassiliicoccales, Methanocellales and Methanosarcinales) that contain the gene mcrA responsible for the methane production (DasSarma et al., 2009; Buan, 2020). Five of the methanogen orders (Methanopyrales, Methanococcales, Methanobacteriales, Methanomicrobiales, and Methanocellales) only contain hydrogenotrophic methanogens, while the Methanomasiliiicoccales group is defined as having obligate methyl-respiring methylotrophic methanogens (Buan, 2020). The Methanosarcinales have the most diverse members, with many genera qualified for more than one methanogenesis pathway, as well as obligate hydrogenotrophic or obligate acetoclastic members (Buan, 2020). See below the biology of the selected groups based on their pathways participation:

Hydrogenotrophic methanogenic archaea: The hydrogenotrophic archaea produce methane from H_2 and CO_2, from formate, or from H_2 and methanol. These archaea use only sodium ions rather than protons for chemiosmotic energy conservation (Thauer et al. 2008). As hydrogenotrophic archaea can use a broad range of substrates it is possible to distinguish them. It has been recognized that the methanogens can be divided into two major groups based on phylogenetic analysis (). They can be classified in Class I methanogens (Methanopyrales, Methanococcales, and Methanobacteriales), as well as most class II methanogens (Methanomicrobiales, Methanocellales, and Methanosarcinales, with the exception of Methanomassiliicoccales). Still, Thauer et al. (2008) have debated that methanogens can be divided into two classses based cytochromes presence or absence, with Methanosarcinales alone possessing cytochromes. The Methanomicrobiales thus belong to the phylogenetic group of Class II methanogens, but to the physiological group of methanogens without cyto-

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chromes. That is why, some reference as Anderson et al. (2009) show that three distinct classes of methanogens: the Class I methanogens, the Methanomicrobiales (Class II), and the Methanosarcinales (Class III).

Here is the classification of hydrogenotrophic archaea in 2 different classes according mainly to Thauer et al. (2008):

Class I

- Methanopyrales: includes only one species, *Methanopyrus kandleri*. This hyperthermophilic species, growing between 84-110 degrees at pH range of 5.5-7.0 is an obligate hydrogenotrophic archea (Sowers, 2019; Kurr et al. 1991, Huber and Stetter, 2001, Liu, 2010).
- Methanococcales: includes organisms that grow exclusively by CO_2 reduction with H_2 or in some cases use formate for growth and methanogenesis. This order includes several mesophilic *Methanococcus spp.*, moderately thermophilic Methanothermococcus spp., and the extremely thermophilic genera *Methanocaldococcus* and *Methanotorris* growing between 20-88 degrees at pH range of 4.5-9.8 (Sowers, 2019).
- Methanobacteriales: includes organisms that grow by CO_2 reduction with H_2, CO, formate and C1-methylated compounds (Angelidaki et al., 2011). *Methanosphaera*, for example, uses H_2 to reduce the methyl group of methanol instead of CO_2. They can grow between a range of 20–88 degrees and 5.0–8.8 ph range (Liu and Whitman, 2008, Thauer et al., 2008).

Class II

- Methanomicrobiales: contains species that differ in their tolerance for salt (range from non halophilic to slightly halophilic), ph (6.1-8.1) and temperature (ranging from 15-60 degrees). Most species grow by CO_2 reduction with H_2, but some species also use formate or secondary alcohols as ethanol, 2-propanol, 2-butanol cyclopentanol (Sowers, 2019; Garcia et al. 2006, Liu and Whitman, 2008, Dianou et al., 2001, Thauer et al. 2008).
- Methanocellales: These species have all been isolated from rice fields (Sowers, 2019) and they grow by CO_2 reduction with H_2 or by using formate in a range of temperature between 25-40 and ph of 6.5-7.8.
- Methanosarcinales (exception of Methanomassiliicoccales): includes the most metabolically diverse species of methanogens. The species grow by CO_2 reduction with H_2, but they can also by methyl reduction with H_2, aceticlastic fermentation of acetate, or methylotrophic catabolism of methanol, methylated amines, and dimethylsulfide (Sowers, 2019). Their growth ranges from temperature of 1-70 and from a pH range of 4.0-10.

Class III

- *Acetoclastic methanogenic archaea:* Acetoclastic methanogens occur in only 2 genera: *Methanosarcina* (also considered hydrogenotrophic or methylotrophic) and *Methanothrix* (only known obligate acetotrophic methanogens also referred previously in the literature as *Methanosaeta*) is well adapted to lower acetate concentration 7-70 μM), when in comparison with *Methanosarcina* species (minimum of ~1.2 mM) (Smith and Ingram-Smith, 2007; Welte and Deppenmeier 2014). On the other hand, *Methanosarcina* is a relative generalist that prefers methanol methylamine to acetate (Boone et al., 2001). Apparently the two genera employ different enzymes to catalyze the first step of acetoclastic methanogenesis while the core steps of methanogenesis are similar in the two genera (Deppenmeier et al., 2002, Smith and Ingram-

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Smith, 2007). The differences between the 2 genera refers to the electron transfer and energy conservation (Smith and Ingram-Smith, 2007). From the conversion of acetyl-CoA to methane and CO2 *Methanosaeta* should earn enough energy to synthesize more than the expended two ATPs, while for *Methanosarcina*, the electrochemical gradient produced by the membrane-bound ECH is used for ATP synthesis during growth on acetate (Smith and Ingram-Smith, 2007). These differences between the two pathways, makes the affinity for the ACS pathway (aceticlastic) of *Methanosaeta* much higher affinity for acetate than the AK-PTA pathway used by *Methanosarcina* (Smith and Ingram-Smith, 2007). Lier et al () also showed in their review that *Methanosarcina* has a relativeliy high umax while *Methanosaeta* are kinetically characterized by a low umax in a wastewater treatments (Figure 2).

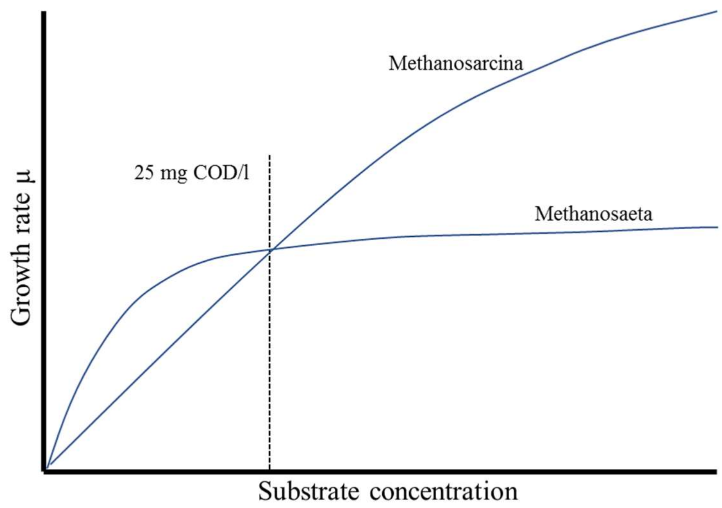

Figure 65: *Curves of the acetotrophic growth of Methanosarcina and Methanosaeta adapated from Lier et al. (2008). The results presented in this figure are according to umax of (5.8 and 1.0 1/d) and the saturation constant (Ks) (30 and 300 mgCOD/l) for Methanosarcina and Methanosaeta, respectively.*

Methylotrophic methanogenic archaea: This archaea can be hydrogen-dependent or hydrogen-independent and they are limited to Methanosarcinales, Methanomassiliicoccales, and one species of Methanobacteriales (Lang et al., 2015). The hydrogen-dependent archaea use a mixed-mode of methanogenesis by combining the hydrogenotrophic and the methylotrophic pathway. These archaea lack both the Wood-Ljungdahl pathway and enzymes consequently they need methylated compounds and hydrogen as electron acceptor and donor, respectively (Kurth et al., 2020; Borrel et al., 2016).

5.1.3 Cultivation of archaea

Initially, the investigation of archaea relied solely on low throughput laboratory cultivation techniques. These cultivation-independent techniques enabled the direct investigation of archaeal ecology and diversity. Cultivation, i.e., growth on specific substrates, remains important as it is the final proof of metabolic activity and is required for detailed physiologic study. Although the majority of microorganisms are not yet cultivable in artificial media as

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pure cultures, the combination of enrichment cultivation and molecular techniques can provide valuable insight into the function of microorganisms, often not possible using uniquely molecular tools.

Similar to the isolation of bacteria, archaeal isolation includes three steps: sample collection, enrichment, and isolation. Compositions of diverse applicable basal media are described in books or scientific articles and further advanced cultivation methods have been utilized such as:

- Anaerobic incubation the Hungate method (Hungate, 1969): A roll tube technique was employed in an attempt to cultivate a maximal portion of the organisms in the gingival crevice area of man. This technique achieves an anaerobic state by flushing the local environment with oxygen-free gas. This technique was important for the cultivation of methanogens due to the anoxic conditions.
- Continuous cultivation: is an open operation system with continuous addition and discharge of the solution in the system. Microorganisms and sterile nutrient solution are added homogeneously to the bioreactor, continuously, while nutrient solution and microorganisms are transformed equivalently in the system. This method can be used to cultivate microbes, especially methanogens (Imachi et al., 2011)
- Ultrafiltration: is one of the most common and simple methods. For example, Nanoarchaeota were physically isolated from their host Ignicoccus spheres by ultrafiltration through 0.45 µm pore-size membranes (Huber et al. 2002).

Anaerobic cultivation methods differ from classic microbiological techniques in several aspects. The instruments are designed to prevent the contact of the cultivated organisms with oxygen as a concentration of 10 ppm is normally lethal, making this field challenging (Wolfe and Metcalf, 2010). For example, isolating methanogenic archaea remains a critical process because of the slow growth of these archaea and because they require 80% H_2 and 20% CO_2 for their optimal growth (Dridi et al., 2011). Because of these specific requirements, laboratories are currently preparing one specific culture medium for each one of the various methanogenic archaea species (Khelaifia et al., 2013). Furthermore, Wolfe (2011) presents a book chapter with basic techniques for the cultivation of methanogenic archaea in anoxic media. Here are we gathered information over cultivation specific to genus associated with their methanogenic pathway:

- Hydrogenotrophic archaea: repeated gassing is necessary because the gaseous substrate in the headspace is consumed according to the specific gas uptake rate and the biomass concentration of gas-utilizing microbes (Hanišáková et al., 2022). Furthermore, they could be cultivated on agar plates without a H_2 atmosphere by using, for example, formate as a substrate (Long et al., 2017). Another strategy suggested by Long et al (2017), is the co-cultivation with bacteria. In this way, pre-substrate (as for example ethanol, butyrate or propionate) is consumed by bacteria and H_2 is gradually released, which is then utilized by hydrogenotrophic methanogens.

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- Aceticlastic archaea: Steinhaus et al (2007) isolated a pure culture of methanogenic *Methanosaeta concilii* with microfluidics technique under N_2/CO_2 atmosphere conditions. Janssen (2003) also described a simple method for the isolation of axenic cultures of members of *Methanosaeta* based on acetone- and isopropanol-utilizing bacteria that slowly ferment substrates to acetate and allows *Methanosaeta spp.* to maintain the acetate concentration at levels below the threshold required for growth of *Methanosarcina spp*.

- Methylotrophic archaea: The same as acetoclastic archaea, methylotrophic species are not dependent on pressure, and it is possible to cultivate them under a H_2-free atmosphere (Hanišáková et al., 2022). Lino et al. (2013) report the methanogenic enrichment culture derived from the sludge of an anaerobic digestion process, that contains a novel methanogenic archaeon as a sole archaeal population.

5.1.4 Culture-independent analysis

With the application of gene-based technologies in microbial ecology, it has become increasingly evident that the diversity of microbial life in natural ecosystems far exceeds that which has been revealed by cultivation-based studies. Culture-independent methods rely on molecular methods to study microbes within their diverse environments. These approaches can give a better insight into the microbial community and its dynamics. Traditional cultivation techniques for the enrichment and isolation of microbes yield only a limited fraction of all microorganisms present. Therefore, molecular tools give an overview about microbial diversity and their relative abundance in a specific community.

Polymerase chain reaction (PCR) amplification provides a fast and sensitive alternative to conventional culture techniques. The technique is on the amplification of specific region of the DNA sequences that can be used to determine the microbial community composition. The small subunit rRNA genes (i.e., 16S rRNA) are regularly employed as phylogenetic marker to describe microbial diversity without the need of cultivation (Dunbar et al., 1999). The structure of the 16S-rRNA genes is defined by an alternation of highly-conserved and hypervariable regions making the 16S sequence an ideal proxy to achieve a trustworthy level of taxonomic information. The conserved regions serve as way for PCR primers to adhere to the sequence while sequencing the variable regions identifies the microorganism. Therefore, the sequences can be used to infer taxonomic identifications based upon bioinformatics alignments against sequence databases (Caporaso et al., 2011). In the last period, the next-generation sequencing (NGS) technology such as the metagenomics have impressively accelerated research by enabling the production of large volumes of sequence data to a drastically lower price per base, compared to traditional sequencing methods. Different NGS platforms were developed and produced a massive amount of sequencing data (up to gigabases and soon even terabases) in parallel. Illumina MiSeq has gained lately in status for use as a platform for analysis of environmental and it provides a lot of data output with short read length, but still several studies have embarked on further refining data analyses for Illumina platforms by improving on methods in library preparation and quality control of sequence reads.

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Molecular techniques have been effectively used for the assessment of methanogens in several environments. The most frequently used molecular marker is the 16S rRNA gene, however for a more specific molecular marker for methanogens, the gene encoding the α subunit of the methyl-CoM reductase (mcrA) was identified and primers were developed for its amplification (Steinberg and Regan, 2009). The *mcrA* gene is thought to be highly conserved and specific for methanogens and Several studies have used this gene as biomarker to determine methanogen community in soils and anaerobic digesters (Liu et al., 2018; Alvarado et al., 2014). Therefore, methanogen phylogenies determined using the mcrA and 16S rRNA genes are largely congruent and both genes have been used to elucidate the diversity and phylogeny of methanogens.

5.1.5 Methanogenic archaea in anaerobic digestion

Anaerobic digestion is a technology known for its potential in terms of methane production through the biological treatment of organic waste and wastewater without input of external electron acceptors (oxygen), offering the potential to reduce treatment cost and to produce energy as 'biogas' (methane) from organic waste. Compared with traditional aerobic treatment, AD has several potential advantages such as lower operational costs from lack of aeration requirements, energy production from biomethane, significantly less biomass production, which reduces handling and disposal costs, and the ability to degrade certain pollutants, which cannot be aerobically removed (Suryawanshi et al., 2010).

During the digestion process, multiple metabolites of high value are synthesized. It is a complex multi-stage process relying on the activity of highly diverse microbial communities including hydrolytic, acidogenic and syntrophic acetogenic bacteria as well as methanogenic archaea. These groups function in syntrophy and have intra-dependent metabolic pathways. Changes in one group can alter this chain of anaerobic process and consequently AD performance (Nguyen et al., 2018). Consequently, the importance of understanding the AD microbiome and the need of establishing microbial indicators of process performance are currently considered as key research subjects towards the improvement of the biomethanation process and the understanding of the process imbalance (Carballa et al., 2015).

The most common disorders of the biogas process are over-acidification (Kleyböcker et al., 2012). This problem occurs due a decline in digestate pH due to the accumulation of fatty acids reflecting a kinetics imbalance between acid production and consumption rate and leading to a breakdown of the reactor buffering capacity (Appels et al., 2011). As a consequence, archaeal communities in AD reactors facing acidification are quickly inhibited, leading to a decreased methane production (Weiland, 2010). Excessive acidification could be avoided by optimising the C/N ratio using a co-digestion system or through the addition of trace elements (Agyeman and Tao, 2014; Kong et al., 2016). Cerón-Vivas et al. (2019) accessed the influence of pH and C/N ratio on methane yield of wastewater and observed a maximal biochemical methane production of 318 L CH4/kgVSS with pH 7.5 and C/N ratio of 8.2±0.18. Alavi-Borazjani et al (2020) have summarized the most common strategies used to control

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excessive acidification in AD within four major categories: (i) the use of additives, (ii) optimisation of the key parameters affecting AD, as for example decrease the organic loading rate (iii) modification of the process configuration, and (iv) co-digestion.

Most of the control in anaerobic digestion is undertaken directly by the microorganisms themselves; however, factors affecting the performances of an anaerobic digester can be divided in three main classes: (i) feedstock characteristics, (ii) reactor design and (iii) operational conditions (Cioabla et al., 2012). Given several advantages, the processing of bioenergy from waste via AD has many severe challenges. Therefore, understanding of the fundamental biological and physicochemical processes in AD is required to improve the technology.

5.1.6 Metabolic pathways

The microbial processes of AD can be described by the sequential steps of hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Each of these steps is accomplished by a group of microorganisms, and it is critical to maintain a balanced reaction rate among the steps or guilds to ensure rapid and stable digestion.

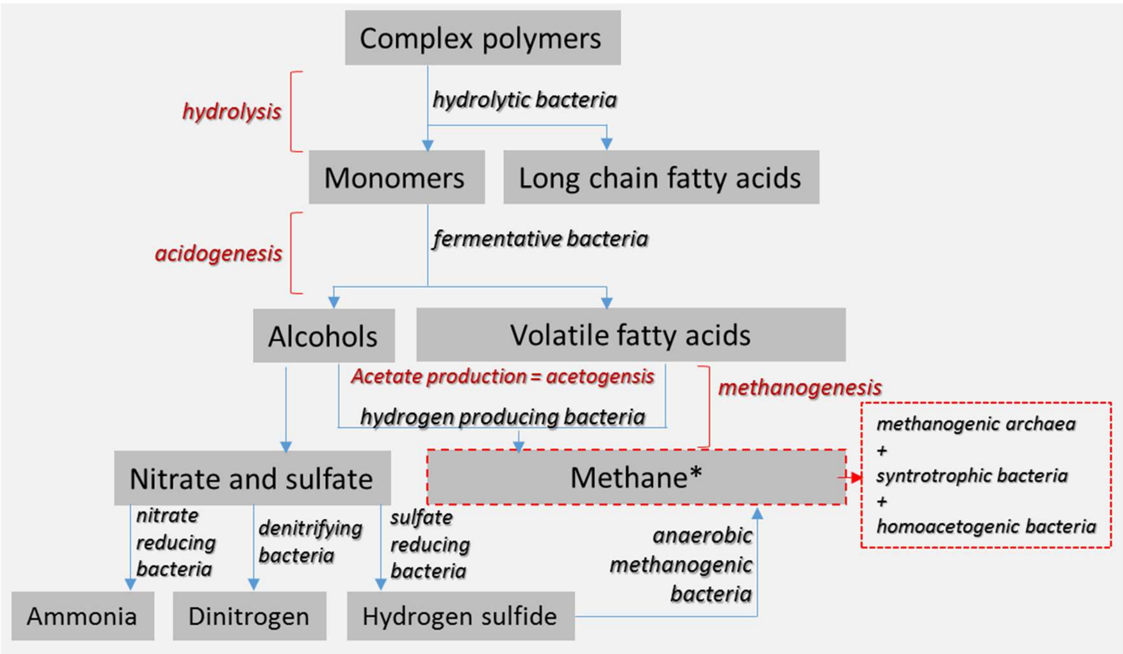

*Figure 66: Main degradation pathways in the anaerobic digestion. *The production of methane has also different pathways not included in this figure where different bacteria and archaea act in combination or with themselves to transform acetate. CO_2 or H_2 in methane.*

Hydrolysis involves the breakdown of polymeric substrates, such as polysaccharides, lipids, and proteins using extracellular enzymes through anaerobic hydrolytic bacteria. These enzymes generally include amylase, cellulase, lipase, pectinase, and protease. These intermediates serve as substrates for the next groups of microorganisms in the process chain. Hydrolytic bacteria are phylogenetically diverse; however, two phyla, namely, Bacteroidetes

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and Firmicutes, are the two most dominant phyla in AD. The abundance of hydrolytic bacteria in AD depends on the type of inoculum, operating temperature, cell retention time and substrate characteristics (Nguyen et al., 2018). From a microbial point of view, the hydrolysis step is not rate-limiting in AD as the hydrolytic bacteria can grow fast and are less sensitive to changes in environmental conditions (Lim et al., 2020).

In the next step of AD there is the acidogenesis where acetogenic bacteria use the products of hydrolyzers bacteria as electron acceptors to generate fermentation products such as formic acid, acetic acid, propionic acid, butyric acid, pentatonic acid, alcohols, CO_2, and H_2 (Nguyen et al., 2018). Bacteroidetes, Chloroflexi, Firmicutes, and Proteobacteria are phyla that contain most identified species of acidogenic bacteria (Stiles and Holzapfel., 1997; Yamada et al., 2006). From the fermentation products, only acetate, formate, H_2, and CO_2, resulted from acidogenesis, can be directly utilized by methanogens for biogas production (Lim et al., 2020). Consequently, in this step, different microbiological steps must be balanced and synchronised, otherwise the process can experience disturbance and accumulation of degradation intermediates such as volatile fatty acids (VFA). This accumulation causes a drop in pH which inhibits or stops methanogenesis completely. The other intermediates can still be used by syntrophic acetogenesis is the process in which these intermediates are further biotransformed to form acetate, H_2, and CO_2 (Venkiteshwaran et al., 2016). The syntrophic fatty-acid oxidizing microbes mainly belong to the genus *Syntrophomonas* (Nguyen et al., 2018). Therefore, the enhancement of syntrophic degradation of propionate is thought as an approach to improving anaerobic digestion efficiency in high ammonia anaerobic digestion processes (Müller et al., 2016). This reaction is dependent on instant H_2 removal by hydrogenotrophic methanogens, with subsequent production of CH_4.

The third stage in AD is acetogenesis, where fermented products by the use of acetogenic bacteria are transformed to acetate, CO_2, and H_2, especially fatty acids and alcohols. These bacteria are called acetogens, non-syntrophic acetogens or homoacetogens and are obligatory sources of H_2 (Hassan et al., 2022). The acetogenesis stepis less favorable in the AD process and the homoacetogenic bacteria are often outcompeted by hydrogenotrophic methanogens for H_2. They are among the most phylogenetically diverse bacterial functional groups and approximately 100 homoacetogenic species have been identified and are phylogenetically classified in 21 different genera (Drake et al. 2006).

In the last step, methane can be produced via three different pathways (hydrogenotrophic, aceticlastic, and methylotrophic) but differ in many aspects of their biochemistry and physiology. The largest phylogenetic diversity is found within the hydrogenotrophic methanogens. They utilize hydrogen as an electron donor for the reduction of carbon dioxide to methane in six steps via the reductive acetyl-CoA or Wood–Ljungdahl pathway (Berghuis et al., 2019). Acetate is directly dismutated to methane and carbon dioxide by acetoclastic methanogens. Only two genera of methanogenic archaea, *Methanosarcina* and *Methanothrix* (former Methanosaeta), are able to grow with acetate as sole energy and carbon source. At conditions that are less favorable for acetoclastic methanogens, syntrophic acetate oxidation may occur. The syntrophic reaction has the same stoichiometry as acetoclastic step; however, the energy produced needs to be shared causing disadvantage to the thermodynamic equilibrium (Thauer et al., 1977; Dyksma et al., 2020). At elevated temperature, and under high

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ammonia conditions, syntrophic acetate oxidation can become the dominant process for acetate consumption (Dolfing, 2014; Wang et al., 2015). During acetoclastic methanogenesis, the acetate carboxyl group is oxidized to CO_2 with the release of electrons that are used to transform the acetate methyl to form methane (Li et al., 2006). Consequently, a complete acetoclastic methanogenesis has no net electron gains or losses.

Methylotrophic methanogens can grow on methylated compounds like methanol or methylamines by dismutation. Typically, this reaction involves four C1 compounds: one in which the methyl group is oxidized to CO_2, while the remaining three methyl groups are reduced to methane by mcr gene (Berghuis et al., 2019). This pathway contributes to a very small amount of methane accumulation comparing with the previous methanogenic pathways (Conrad, 2005).

Direct interspecies electron transfer (DIET) is also an effective form of syntrophy in methanogenic communities. In this step, electrons transfer directly from electron donors to electron acceptors with fewer biological enzymatic reactions (Summers et al., 2010). The bacteria involved in DIET include mainly include *Desulfobacula, Desulfobacterium, Deferribacter, Geobacter sp., Geoalkalibacter, G. metallireducens, G. sulfurreducens, Thauera sp., Syntrophus sp., Pseudomonas sp*, and *Methanothrix sp*. (van Steendam et al. 2019). DIET suggest to be a new way to improve anaerobic digestion performance and there are few reviews on comprehensively summarizing the mechanisms of DIET and the effects of DIET mediated by conductive materials on anaerobic digestion performances (Gahlot et al., 2020; Nguyen et al., 2021). Therefore, details about unclear biological mechanisms, influences of non-DIET mechanisms, limitations of organic matters syntrophically oxidized by way of DIET, and problems in practical application need to be further researched.

5.1.7 Symbiosis and synthrophy

In the anaerobic digestion, hydrolytic and fermentative bacteria, syntrophic bacteria, and methanogenic archaea associate via intricate symbiosis to allow the volumetric reduction and conversion of organic waste to methane (Kleinsteuber, 2018). Saha et al (2020), reviewed the microbial symbiosis during anaerobic biomethanation followed by alteration in microbial communities in AD due to the feeding of various types of organic waste, and the impact of microbial alterations on interspecies networks and biomethanation. In this review, the authors showed that all steps are followed by each other in a symbiotic work (Saha et al., 2020). Substrates (as sugars, glycerol, and amino acids) produced by hydrolytic microorganisms are used in acidogenesis by fermentative bacteria (Saha et al., 2020). Besides this metabolic cooperation providing trophic benefits, there exists a commensalistic symbiosis of hydrolytic microorganisms with some host eukaryotes that have anaerobic conditions for instance the rumen of ruminants (Wrede et al. 2012). Therefore, maybe similar microbes can act in interaction in anaerobic digestion.

The products from acidogenic pathways (organic acids, short-chain fatty acids, alcohols, H_2, and CO_2) are used by different metabolic metabolisms (Saha et al., 2020). While the long-chain fatty acids are oxidized to acetate and hydrogen by acetogenic bacteria, the acids can

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be oxidized into acetate via syntrophic fatty acid oxidizers and syntrophic acetate oxidizers act on oxidizing acetate into H_2 and CO_2 (Saha et al., 2020). Another symbiotic relationship during acidogenesis exists between lactic acid bacteria and butyrate-producing bacteria where the butyrate-producing bacteria form more butyrate from lactate and acetate culminates in a higher yield of hydrogen (Detman et al. 2019; Mutungwazi et al., 2021). Due to the several associations present in the acidogenesis, it is visible that this is a product-determining step in AD processes where control and optimization strategies should be clarified.

Then, in the acetogenesis step occurs the conversion of the products of acidogenesis into acetate and H_2 by acetogenic bacteria and obligate H_2-producing bacteria. The gas H_2 formed in the acetogenesis step can inhibit the action of acetogenic bacteria. However, the hydrogenotrophic bacteria in the methanogenesis step can consume and convert the H_2 into methane. Mutungwazi et al. (2021) mention that there is a beneficial syntrophy between the acetogenic and hydrogenotrophic methanogens and that it is worthwhile to establish H_2 inhibition levels for different substrates through a profiling of the taxonomy and functional roles in the AD. Lastly, the methanogenesis step has 3 different routes. The survival of methanogens is largely dependent on the acetogens and acidogens as the conversion of simple monomers into VFAs, acetic acid, carbon dioxide and hydrogen depend on these two groups.

In anaerobic digestion, syntrotrophy usually refers to the associated relationship between organisms based upon H_2 transfer from one to the other, and an H_2-producing organism that relies on H_2-consuming organisms for its growth. Kamagta (2015), for example, shows a list of different bacteria and reactions involved in the degradation of low-molecule organic matter, syntrophic associations and bacteria in the methanogenesis. Sometimes this term could be mixed with symbiosis (Johnravindar et al., 2021). Besides, the microbial ecology revolving around syntrophy is still poorly understood because only a few syntrophs have been isolated (Schink and Stams, 2013). To elucidate the complex syntrophic acid degrading ecology, it is essential to investigate who participates in syntrophy in situ and how other organisms may interact with syntrophs to help stabilize acid degradation.

5.1.8 Bioaugmentation of methanogenic archaea

Bioaugmentation is a strategy to introduce microorganisms with desirable properties into a biological system to increment specific microbial functions of interest. With that, bioaugmentation could accelerate the start-up and maintain the stability of bioreactors and enhance the conversion rate of complex substrates. Archaea are slow-growers with low biomass yields that produce explosive by-products. Nevertheless, because of the important and limiting role of archaea in anaerobic digestion, it is valuable to explore the potential impact of an improved archaeal consortium on anaerobic digestion as a first step before more thought can be given towards specific augmentation concepts.

In recent years, bioaugmentation has also been applied to increase the biogas production of AD (Li et al., 2018; Nielsen and Angelidaki, 2008). As an example, the bioaugmentation of anaerobic digestion communities by the adapted hydrolytic consortia increased the biogas

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yield by 10–29% in the anaerobic digestion of maize silage (Poszytek et al., 2017). Therefore, the technique of involving enriched or mixed cultures of allochthonous or indigenous microorganisms to enhance a required biological activity in the system investigated can improve the process performance of AD.

Several studies show that bioaugmentation has being used to alleviate ammonia related problems in bioreactors (eg. Yan et al., 2022). Tian et al (2019) showed that bioaugmentation through an enriched culture, and a mixed culture composed 50/50 by *Methanoculleus thermophilus* and the enriched culture improved methane yield and decreased the volatile fatty acids. In the same line, bioaugmentation with certain strains the SAO *Clostridium ultunense* and the hyrogenotrophic archea *Methanoculleus* has been proven to be an effective way to alleviate ammonia inhibition (Fotidis et al. 2014). Furthermore, Yang et al (2019) used different microorganisms (including obligate aceticlastic methanogen, facultative aceticlastic methanogen, hydrogenotrophic methanogen and syntrophic acetate oxidizing bacteria) on anaerobic digestion to mitigate the ammonia inhibition. The authors concluded that bioaugmentation with hydrogenotrophic methanogen *Methanobrevibacter smithii* together was the optimal choice for the methane production (Yang et al., 2019). Besides ammonia limitation, Nzila et al. (2016) shows in the review many other bioaugmentation strategies and inocula to overcome many other problems found in bioreactors such as low temperature limitation, toxicity induced by O_2 and recovery time following overload through bioaugmentation with either single or mixed microorganisms to increase in biogas formation.

Nonetheless, not all bioaugmentation cases result in a positive impact on digestion performance. Despite the merits of bioaugmentation, the main concern regarding bioaugmentation arises as well. In one example, in a continuous AD system, the injected bioaugmentation is probably washed out ultimately due to the daily fed-withdraw mode (Romero-Güiza et al., 2016). Yang et al. (2019) shows a list of different publication with failure of bioaugmentation. From the methanogens, methanogens of *Methanosarcina* have proven to be more robust and appropriate in bioaugmentation to increase methanogenesis phase (Yang et al., 2019).

5.2 Bacteria

5.3 Eukaryotes

While prokaryotic communities in anaerobic digestion are commonly studied, the eukaryotic community is poorly known. Furthermore, most research focusses on microscopic determination or first generation sequencing (Priya et al., 2008a; Priya et al., 2008b; Bhaskaran et al., 2016; Matsubayashi et al., 2017). Research found that, out of the total number of microbial rRNA in anaerobic digestion, 0.1 – 1.4% accounted for eukaryotes. Although the number of eukaryotes cannot precisely be determined because of the heterogeneous rRNA gene copy numbers, it shows that there is a eukaryotic community in anaerobic digestion (Matsubayashi et al., 2017).

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5.3.1 Anaerobic fungi

Biology of anaerobic fungi: Anaerobic fungi of the phylum *Neocallimastigomycota* mainly inhabit the digestive system of mammals and reptiles with a fibrous diet. They have been found worldwide and it appears that host phylogeny is more important than geographical location (Haitjema et al., 2014; Gruninger et al., 2014). Despite their relatively low abundance in these animals, they are one of the key players in degradation of lignified materials, where they colonize the fresh feed within minutes. Mitochondria are completely absent in anaerobic fungi. For respiration, they use mitochondria-related organelles named hydrogenosomes. Anaerobic fungi perform mixed acid fermentation and monosaccharides are fermented into hydrogen, carbon dioxide, formate, acetate, lactate, ethanol and succinate (**Figure 67**) (Haitjema et al., 2014; Gruninger et al., 2014; Hackstein et al., 2019; Vinzelj et al., 2019).

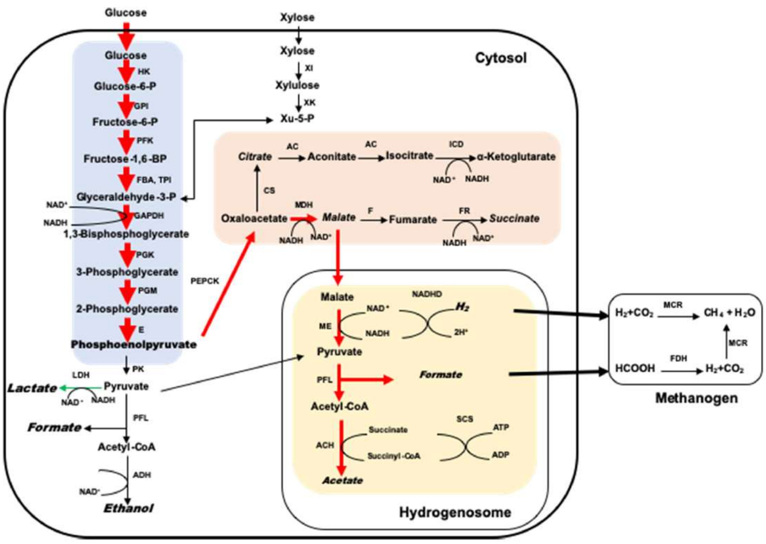

Figure 67: Metabolic pathway for glucose and xylose in anaerobic fungi, as proposed by Li, et al. (2021). A complete explanation of all enzymes involved can be found in the same paper.

Anaerobic fungi excrete a large range of fiber-degrading enzymes, including cellulases, hemicellulases, pectinases, esterases, glycoside hydrolases, carbohydrate esterases, glycosyl transferases, polysaccharide lyases, carbohydrate esterases, and carbohydrate-binding modules. Together with their ability to penetrate plant tissues with their rhizoids, anaerobic fungi can effectively degrade fibers (Haitjema et al., 2014; Cheng et al., 2018).

Cultivation of anaerobic fungi: Anaerobic fungi are obligate anaerobes, and are usually cultivated stationary in a liquid medium at 39 °C, but growth is observed between 33 and 41 °C (Wilken et al., 2019). Generally, they are grown in Hungate tubes on either soluble or insoluble carbons source, with 100% CO_2. Overgrown substrate is preferred as inoculum, as it reduces lag time in comparison to inoculation with free zoospores. Another growth technique is with agar roll tubes. Reducing agent such as sodium sulfide or L-cysteine hydrochloride are added to ensure a negative redox level. The redox indicator resazurin is used to check the redox level (Haitjema et al., 2014).

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Anaerobic fungi can be isolated directly from rumen, but it has become clear that spores can also be obtained from feces. They are isolated by making a serial dilution of an anaerobic fungi-containing material and inoculate that in an antibiotic-containing growth medium with an insoluble carbon source such as grass, hay, or wheat straw. Growth is observed either by gas production or visible biomass growth (Haitjema et al., 2014).

Culture-independent analysis: Molecular-method-based analysis of anaerobic fungi raises problems, as anaerobic fungi have the lowest GC value of all sequenced fungi, with an overall average of down to 25% GC, where non-coding region can have down to 16% GC (Wilken et al., 2019).

Previously, the 18s rRNA gene was mostly used for anaerobic fungi phylogeny, but this gene is highly conserved, making it unusable for determine below order level. Therefore, alternatives such as the ITS1 (internal transcribed spacer 1) and the D1/D2 region of the large ribosomal subunit (LSU) have been developed. At this moment, the ITS1 region remains the most used in fungal phylogeny (Vinzelj et al., 2019; Edwards et al., 2017).

Three sets of primers were developed by Dollhofer, et al. (2016), to study the quantity, diversity and activity of anaerobic fungi in anaerobic digestion. In an anaerobic digester fed on cattle slurry and maize silage, they used qPCR primers on the 18S rRNA gene and found up to 10^9 rRNADNA copies/ml of anaerobic fungi (Dollhover et al., 2017). The correlation of DNA copy numbers and cell biomass, however, is complicated as it is influenced by factors such as growth phase. For phylogeny, they used a primer paired targeting the large ribosomal subunit (LSA, 28s rRNA). They found the genera *Caecomyces*, *Piromyces*, and *Anaeromyces*. Lastly, they developed a primer pair for quantification of anaerobic fungi glycoside hydrolases family 5 endoglucanases mRNA. Although it could show transcription in growing anaerobic fungi cultures, they could not detect it in a biogas plant operating at 40°C (Dollhofer, et al.; 2016). In a later study, they found 10^2gene copies per ml, whereas they found levels up to 10^5 in rumen fluid (Dollhover et al., 2017).

Anaerobic fungi in anaerobic digestion: Although the presence and long-time survival of anaerobic fungi in digesters is not often studied, it is known that they are present in digesters fed with manure (Dollhover et al., 2017). However, it has been reported that anaerobic fungi were not found in anaerobic sludge digesters (Matsubayashi et al., 2017; Hirakata et al., 2019).

Using the same primers discussed in the previous section, Dollhofer, et al. (2017) studied the presence and activity of anaerobic fungi in biogas plants. They found that up to 10^9rRNA gene copies/ml were found in biogas plants fed with cattle manure or slurry. Because presence of anaerobic fungi doesn't mean that they are metabolically active, the presence of anaerobic fungi GH5 endoglucanase mRNA was also measured as an indication of cellulose degradation activity. It was found that only two biogas plants showed transcriptional activity, with the highest having only $1.8x10^2$ transcripts/ml, which is 10^3-fold lower than what they found in cattle rumen (Dollhofer, et al.; 2016). Furthermore, they could only isolate one anaerobic fungi of the genus *Piromyces* from all biogas plants (Dollhover et al., 2017). This indicates that the anaerobic fungi get imported with the fresh manure, but they are not able to settle in the anaerobic digester.

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Symbiosis and syntrophy: It has been found that anaerobic fungi can form stable co-cultures with methanogenic archaea in vitro, whereby the fungi's metabolites are consumed by the archaea. Research found that acetate and hydrogen production were upregulated, while the production of ethanol and lactate were downregulated in an anaerobic fungi in co-culture with a methanogen. Acetate and hydrogen are the main substrates for methanogens (Li et al., 2021).

Bioaugmentation of anaerobic fungi: The recalcitrance of lignocellulosic material is one of the rate-limiting steps in anaerobic digestion. Multiple studies show the potential of a co-culture of an anaerobic fungus with a methanogen in degrading lignocellulosic materials, including paper, fiber, leafs, cellulose, ryegrass stem, rice straw, wheat straw, corncob, and corn core (Cheng et al., 2018; Li et al., 2020; Li et al., 2021), where biogas production was either accelerated or increased. However, most of these researches focus on aseptic systems, whereas an anaerobic digester contains a rich diversity of microorganisms.

Prochazka, et al. (2012) performed experiments with anaerobic sludge, and a complex carbon source, with the addition of 0,1g/l dry mycelium of the anaerobic fungi *Anaeromyces* spp. An increase in biogas production was observed. Although a relatively large amount of mycelium was added, the anaerobic fungi could not be detected after 7 days, indicating that the fungi could not settle in the anaerobic reactor (Prochazka, et al. (2012).

Akyol, et al. (2019) studied the ability of *Orpinomyces* sp. to increase biogas production from various lignocellulosic materials with anaerobic seed sludge or cow manure. They found that, in all cases, methane production was both accelerated and increased, and cellulose loss was increased. Furthermore, they found that the bacterial community was heavily influenced by the fungal bioaugmentation, whereby the augmentated trials showed a significantly lower diversity. This shows that addition of an anaerobic fungi affects the process (Akyol et al., 2019).

Experiments performed by Dollhofer, et al. (2018) with bioaugmentation of anaerobic fungi *Neocallimastix frontalis* in methane production of lignocellulosic biomass, showed that biogas production was increased and accelerated after hydrolytic pretreatment. However, anaerobic fungi activity and viability decreased over time (Dollhover et al., 2018).

In many studies, the long-term survival of anaerobic fungi in anaerobic fermenters is rarely considered. For bioaugmentation in an agricultural biogas plant, establishment of the anaerobic fungi in the anaerobic digester would be the most ideal case. Otherwise, the fungi must be added at periodic intervals.

5.3.2 Other eukaryotes

With anaerobic fungi as the best studied eukaryotes in anaerobic digestion, other eukaryotes have been greatly overlooked.

Many anaerobic protozoa are known to contain endosymbiotic methanogens (Embley and Finlay, 1993; Fenchel and Finlay, 2018). Mostly found in ciliates, they are also occasionally

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present in other eukaryotes such as amoebes and flagellates. The basis of the symbiotic relationship between ciliates and methanogenic archaea lies in the ciliate's metabolism. Instead of mitochondria, ciliates have hydrogenosomes, where they ferment pyruvate into acetate and hydrogen gas. Without methanogenic endosymbionts, this hydrogen gas can accumulate into concentrations high enough to thermodynamically inhibit pyruvate fermentation. In the symbiosis with methanogens, the hydrogen is consumed quickly and the fermentation reaction is feasible (**Figure 64**). The number of methanogens in ciliates varies, with 300-400 in *Metopuspalaeformis*to 6,000-10,000 in *Metopuscontorus*, but with a relatively constant total volume of approximately 2% of the host. The methanogens and hydrogenosomes are disk shaped and stacked with alternating methanogens and hydrogenosomes, ensuring a close physical contact **(Figure 65)** (Fenchel and Finlay, 2018). On the significance of this relationship, Fenchel and Finlay write:

"Taking into consideration (1) that methanogenic symbionts do not apparently occur as free living, (2) that they have many special adaptation to life as endosymbionts, and (3) that aposymbiotic ciliates apparently cannot be re-infected with methanogens, it seems to indicate that the endosymbiotic methanogens have approached the status of organelles." (Fenchel and Finlay, 2018)

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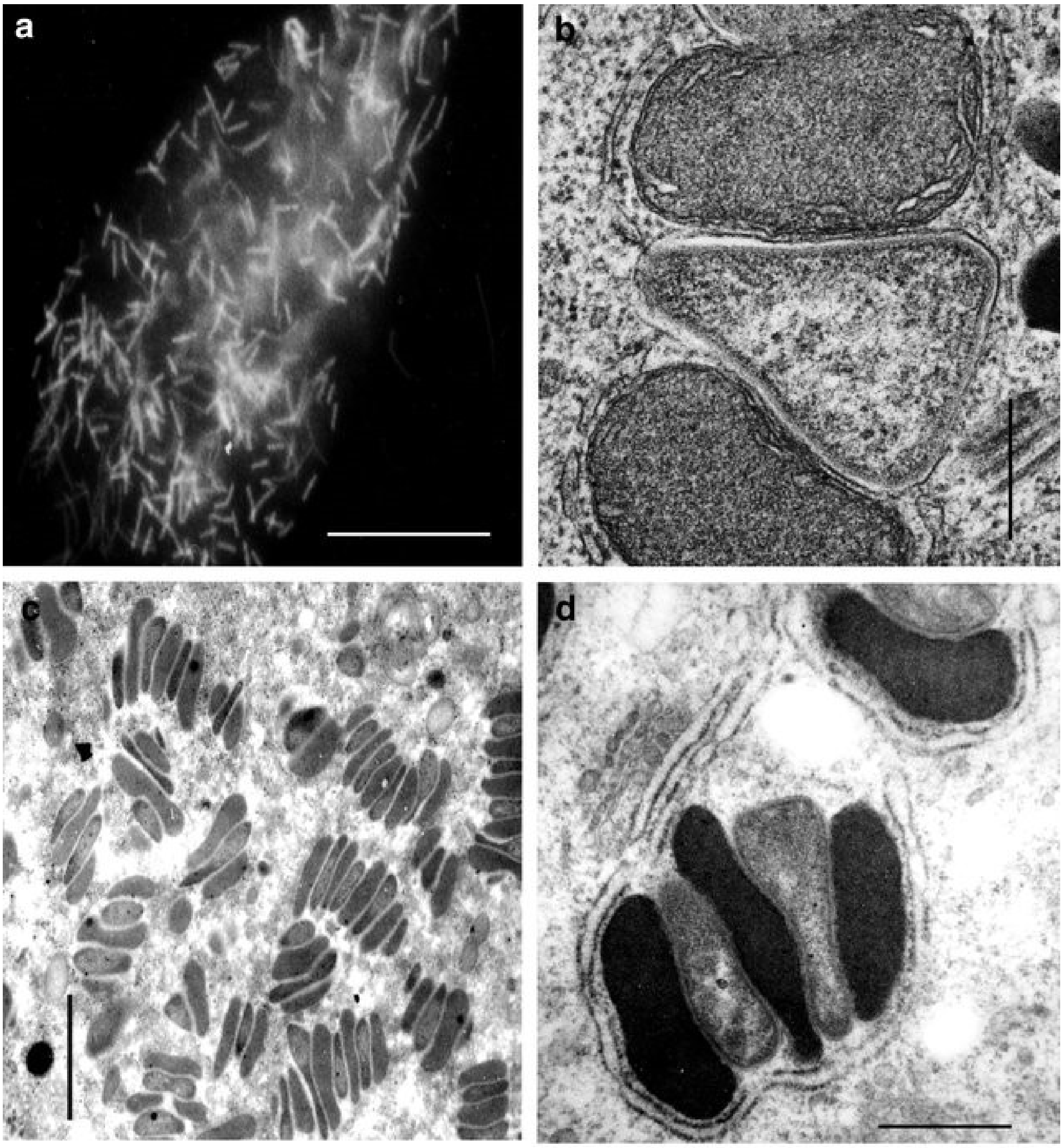

Figure 68: Fluorescence of methanogens in Metopuspalaeformis; scale bar: 10 mm. (b) A methanogen sandwiched between two hydrogenosomes in Metopuscontortus; scale bar: 0.5 mm. (c, d) Stacks of alternating hydrogenosomes (darker) and methanogens in Plagiopylafrontata: scale bars 5 and 0.5mm, respectively. Figure adjusted from Fenchel & Finlay (2018).

The quantitative role of endosymbiotic methanogens in protozoa in methane production in natural habitats is estimated relatively low at 3%. In environments rich with sulphate and organic matter, the role is bigger, with experimental values of 20-80% (Fenchel and Finlay, 2018). However, the role of protozoa with endosymbiotic methanogens in anaerobic digestion has not yet been studied.

From 5 anaerobic digester samples, Matsubayashi, et al. (2017) cloned 164 eukaryotic 18s rRNA genes. After sequencing, they found 66% fungi, 14% Alveolata, 11% Viridiplantae, 6% metazoan, 1% amoebozoa, 1%rhizaria, and 1% Stramenopiles. An interesting group they

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found were the Cryptomycota or Rozellomycota (Matsubayashi, et al., 2017). This is a fungi, or fungal-related group of organisms, where not a lot is known about, hence the name Cryptomycota (Lara et al., 2009). They have been found in multiple environments such as soil, freshwater sediment, marine sediment, peat bogs, and anaerobic digesters (Gleason et al., 2012; Lazarus and James, 2015; Qin et al., 2021), but their ecological role remains unclear.

In a study done on a lab-scale UASB, Priya et al. (2008a) studied the community dynamics of protozoa by observation and determination by microscope. They could identify the ciliates *Metopus*, *Cyclidium* and *Colpoda*, and the flagellates *Menoidium*, *Rhyncomonas*, and *Bodo* on genus level. Correlations of up to R^2 = 0.9158 between gas production and protozoa concentration were found. They propose that this effect might come due to excretion of hydrolytic enzymes, or methane production by endosymbiotic methanogens (Prabhakaran et al., 2016).

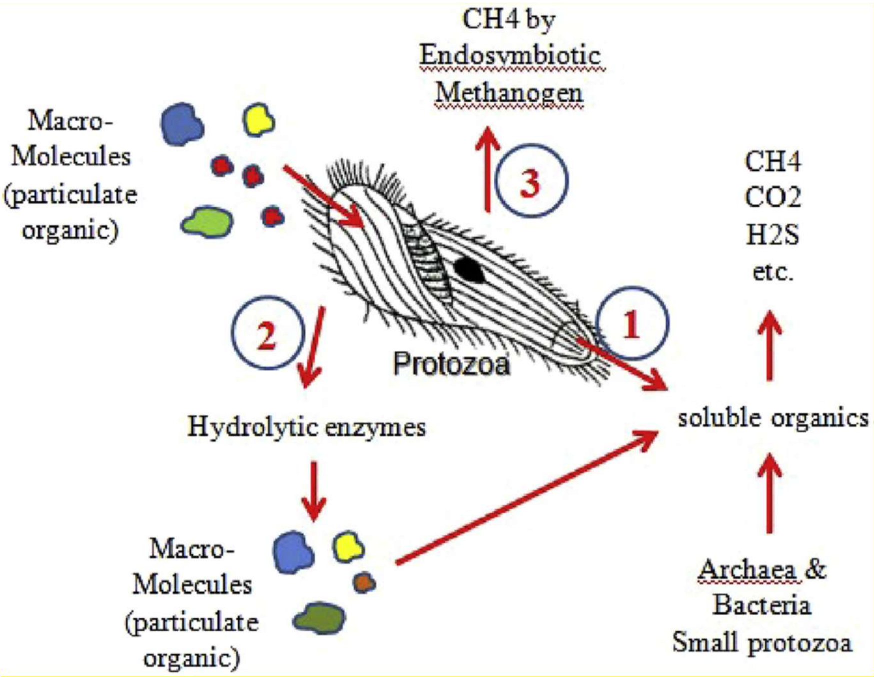

Figure 69: Hypothetical scheme: the role of protozoa in anaerobic digestion of complex organics. Figure from Prabhakaran, et al. (Prabhakaran et al., 2016).

In a similar experiment by Priya, et al. (2008a), in a lab-scale continuous stirred tank anaerobic reactor fed with sodium acetate or sodium oleate, it was found that during the start-up phase, up to 10^6 protozoa ml^{-1} were observed, mostly ciliates and flagellates. A correlation between COD degradation and ciliate density was found (Priya et al., 2008a).

It is clear that the research of eukaryotes in anaerobic digestion is still in its infancy and that many blind spots exist. The two methods, determination by microscopic observation and next-generation sequencing, both have their drawbacks. The determination of eukaryotes based on microscopic observation is time-intensive and sensitive for observer bias. Next generation sequencing, however, has a primer bias (Kounosu et al., 2019) and often an insufficient database.

Next to that, taking in account the close genetic relationship between free-living and endosymbiotic methanogens (Beinart et al., 2017), NGS cannot discriminate between the two

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groups methanogens. It must be mentioned that the studies working with microscopic determination also did not study the endosymbionts.

References

- Alavi-Borazjani SA, Capela I, Tarelho LAC. 2020. Over-acidification control strategies for enhanced biogas production from anaerobic digestion: A review. Biomass and Bioenergy. 143:105833
- Alvarado A, Montañez-Hernández LE, Palacio-Molina SL, Oropeza-Navarro R, Luévanos-Escareño MP, Balagurusamy N. 2014. Microbial trophic interactions and mcrA gene expression in monitoring of anaerobic digesters. Front Microbiol. 12
- Agyeman FO, Tao W. 2014. Anaerobic co-digestion of food waste and dairy manure: Effects of food waste particle size and organic loading rate. J Environ Manage. 133, 268-274.
- Angelidaki I. Karakashev D, Batstone DJ, Plugge CM, Stams AJM. 2011. Biomethanation and Its Potential. Methods in Enzymology, Volume 494.
- Akyol Ç, Ince O, Bozan M, Özbayram G, Ince B. 2019. Fungal bioaugmentation of anaerobic digesters fed with lignocellulosic biomass: What to expect from anaerobic fungus Orpinomyces sp.. Bioresour Technol. 277. 1-10.
- Appels L, Assche AV, Willems K, Degrève J, Van Impe J, Dewil R. 2011. Peracetic acid oxidation as an alternative pre-treatment for the anaerobic digestion of waste activated sludge, Bioresour Technol. 102, 4124–4130.
- Beinart RA, Rotterová J, Cepicka I, Gast R, Edgcomb V. 2017. The genome of an endosymbiotic methanogen is very similar to those of its free-living relatives. Environ Microbiol. 20, 2538-2551.
- Berguis BA, Yu FB, Schulz F, Quake SR. 2019. Hydrogenotrophic methanogenesis in archaeal phylum Verstraetearchaeota reveals the shared ancestry of all methanogens. PNAS. 116, 5037-5044.
- Bomberg M, Montonen L, Münster U, German NJ. 2008. Diversity and function of archaea in freshwater habitats. Curr. Trends Microbiol., 4, 61–89.
- Boone DR, Mah RA. 2001. Genus I. Methanosarcina Kluyver and van Niel 1936, 400,AL emend. Mah and Kuhn 1984a, 266 (Nom. Cos., Opinion 63 of the Jud. Comm. 1986b, 492), p. 269-276. In D. R. Boon and R. W. Castenholz (ed.), Bergey's manual of systematic bacteriology second edition, The Archaea and the deeply branching and phototrophic Bacteria, 10 vol. 1. Springer.
- Borrel G, Adam PS, Gribaldo S. 2016. Methanogenesis and the Wood–Ljungdahl pathway: An ancient, versatile, and fragile association. Genome Biol. Evol., 8, 1706–1711.
- Buan, N., 2020. Emerging Topics in Life Sciences. 2, 629–646
- Cabello P, Roldán MD, Moreno-Vivián C. 2004. Nitrate reduction and the nitrogen cycle in archaea. Microbiology (Reading), 150, 3527-3546.
- Caporaso, JG, Lauber CL, Walter WA. Berg-Lyons D, Lozupone CA et al. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA. 108, 4516–4522.
- Carballa M, Regueiro L, Lema Jm. 2015. Microbial management of anaerobic digestion: exploiting the microbiome-functionality nexus. Curr Opin Biotech. 33, 103–111.
- Cheng Y, Shi Q, Sun R, Liang D, Li Y, Li Y, Jin W, Zhu W. 2018. The biotechnological potential of anaerobic fungi on fiber degradation and methane production. World J Microbiol Biotechnol. 34, 155.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Cioabla AE, Ionel I, Dumitrel GA, Popescu F, 2012. Comparative study on factors affecting anaerobic digestion of agricultural vegetal residues. Biotechnol Biofuels 5, 39.
- Conrad R. 2005. Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and a proposal. Organic Geochemistry. 36, 739–752.
- Dassarma S, Coker J, Dassarma P. 2009. Archaea (overview). Encyclopedia of Microbiology (Third Edition), 1-23.
- Deppenmeier U, Johann A, Hartsch T, Merkl R, Schmitz RA, et al. 2002. The genome of Methanosarcina mazei: evidence for lateral gene transfer between bacteria and archaea. J Mol Microbiol Biotechnol. 4, 453-61.
- Detman A, Mielecki D, Chojnacka A, Salamon A, Błaszczyk MK, Sikora A. 2019. Cell factories converting lactate and acetate to butyrate: Clostridium Butyricum and microbial communities from dark fermentation bioreactors. Microb Cell Factories. 18, 1–12.
- Dianou D, Miyaki T, Asakawa S, Morii H, Nagaoka K, Oyaizu H, Matsumoto S. 2001. *Methanoculleus chikugoensis* sp. nov., a novel methanogenic archaeon isolated from paddy field soil in Japan, and DNA-DNA hybridization among Methanoculleus species. Int. J. Syst. Evol. Microbiol. 51, 1663–1669.
- Dollhover V, Callaghan TM, Dorn-In S, Bauer J, Lebuhn M. 2016. Development of three specific PCR-based tools to determine quantity, cellulolytic transcriptional activity and phylogeny of anaerobic fungi. J Microbiol Methods. 127, 28-40.
- Dollhover V, Callaghan TM, Griffith GW, Lebuhn M, Bauer J. 2017. Presence and transcriptional activity of anaerobic fungi in agricultural biogas plants. Bioresource Technology. 235, 131-139.
- Dollhofer V, Dandikas V, Dorn-In S, Bauer C, Lebuhn M, Bauer J. 2018. Accelerated biogas production from lignocellulosic biomass after pre-treatment with Neocallimastix frontalis. Bioresource Technology. 264, 219-227.
- Dolfing J. 2014. Thermodynamic constraints on syntrophic acetate oxidation. Appl Environ Microbiol. 80, 1539–41.
- Dombrowski N, Seitz, KW, Teske AP, Baker BJ. 2017. Genomic insights into potential interdependencies in microbial hydrocarbon and nutrient cycling in hydrothermal sediments. Microbiome. 5, 106.
- Dong Y, Shan Y, Xia K, Shii L. 2021. The Proposed Molecular Mechanisms Used by Archaea for Fe(III) Reduction and Fe(II) Oxidation. Front. Microbiol. 12: 690918.
- Drake HL, Küsel K, Matthies C. 2006. Acetogenic Prokaryotes. The Prokaryotes, Springer, New York, 354–420.
- Dridi B, Raoult D, Drancourt M. 2011. Archaea as emerging organisms in complex human microbiomes. Anaerobe. 17, 56–63.
- Dunbar J, Tsakala S, Barns SM, Davis JA, Kuske CR. 1999. Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. Appl. Environ. Microbiol. 65, 1662–1669.
- Dyksma S, Jansen L, Gallert C. 2020. Syntrophic acetate oxidation replaces acetoclastic methanogenesis during thermophilic digestion of biowaste. Microbiome. 8, 105.
- Edwards JE, Forster RJ, Callaghan TM, Dollhofer V, Dagar SS. 2017. PCR and Omics Based Techniques to Study the Diversity, Ecology and Biology of Anaerobic Fungi: Insights, Challenges and Opportunities. Front Microbiol. 8:1657.
- Embley T, Finlay B. 1993. Systematic and morphological diversity of endosymbiotic methanogens in anaerobic ciliates. Antonie Van Leeuwenhoek. 64, 261-271.
- Fenchel T, Finlay B. 2018. Free-Living Protozoa with Endosymbiotic Methanogens. Microbiology Monographs, vol 19. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-13615-3_1

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Fotidis IA, Wang H, Fiedel NR, Luo G, Karakashev DB, Angelidaki I. 2014. Bioaugmentation as a Solution To Increase Methane Production from an Ammonia-Rich Substrate. 612 Environ Sci Technol. 48, 7669-7676.
- Garcia JL, Ollivier B, Whitman W. 2006. The order methanomicrobiales. In "The Prokaryotes, Vol. 3," (M. Dworkin, ed.), 208–230. Springer, New York.
- Gahlot P, Ahmed B, Tiwari SB, Aryal N, Khursheed A et al. 2020. Conductive material engineered direct interspecies electron transfer (DIET) in anaerobic digestion: mechanism and application. Environ. Technol. Innov. 20:101056.
- Gleason F, Carney L, Lilje O, Glockling S. 2012. Ecological potentials of species of Rozella (Cryptomycota). Fungal Ecol. 5. 651–656.
- Gruninger RJ, Puniya AK, Callaghan TM, Edwards JE, Youssef N. et al. 2014. Anaerobic Fungi (Phylum Neocallimastigomycota): Advances in understanding of their taxonomy, life cycle, ecology, role, and biotechnological potential. FEMS Microbiology Ecology. 90, 1-17.
- Hackstein JHP, Baker SE, van Hellemond JJ, Tielens AGM. 2019. Hydrogenosomes of Anaerobic Fungi: An Alternative Way to Adapt to Anaerobic Environments. In: Tachezy J, (eds.). Hydrogenosomes and Mitosomes: Mitochondria of Anaerobic Eukaryotes. Microbiology Monographs, vol 9. Springer, Cham. https://doi.org/10.1007/978-3-030-17941-0_7
- Haitjema CH, Solomon KV, Henske JK, Theodorou MK, O'Malley MA. 2014. Anaerobic Gut Fungi: Advances in Isolation, Culture, and Cellulolytic Enzyme Discovery for Biofuel Production. Biotechnol Bioeng. 111, 1471-1482.
- Hanišáková N, Vítězová M, Rittmann SKMR. 2022. The Historical Development of Cultivation Techniques for Methanogens and Other Strict Anaerobes and Their Application in Modern Microbiology. Microorganisms. 10, 412.
- Hassan et al., 2022. Eco-friendly biogas production from algal biomass. Handbook of Algal Biofuels Aspects of Cultivation, Conversion, and Biorefinery, 225-249.
- Hirakata Y, Hatamoto M, Oshiki M, Watari T, Kuroda K, Araki N, Yamaguchi T. 2019. Temporal variation of eukaryotic community structures in UASB reactor treating domestic sewage as revealed by 18S rRNA gene sequencing. Sci Rep. 9:12783.
- Huber and Stetter, 2001. Order I. Methanopyrales ord. nov. In "Bergey's Manual of Systematic Bacteriology, Vol. 1," (D. R. Boone, R. W. Castenholz, and G. M. Garrity, eds.), second ed., pp. 353–355. Springer, New York. (The Archaea and the Deeply Branching and Phototrophic Bacteria).
- Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC, Stetter KO. 2002. A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. Nature. 417, 63–67.
- Hungate, 1969. A roll-tube method for cultivation of strict anaerobes. Methods Microbiol. 3B, 117–132.
- Iino T, Tamaki H, Tamazawa S, Ueno Y, Ohkuma M, et al. 2013. *Candidatus Methanogranum caenicola*: a novel methanogen from the anaerobic digested sludge, and proposal of Methanomassiliicoccaceae fam. nov. and Methanomassiliicoccales ord. nov., for a methanogenic lineage of the class Thermoplasmata. Microbes Environ.;28 in press.
- IPCC (2007). "Climate Change 2007: the Physical Science Basis," in Proceedings of the Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, eds D. Qin, S. Solomon, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, et al. (Cambridge: Cambridge University Press).
- Imachi I, Aoi K, Tasumi E, Saito Y, Yumanaka Y, et al., 2011. Cultivation of methanogenic community from subseafloor sediments using a continuous-flow bioreactor. ISME. 5, 1913–1925.
- Janssen PH. 2003. Selective enrichment and purification of cultures of *Methanosaeta* spp. J Microbiol Methods. 52, 239-44.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Johnravindar D, Patria RD, Lee JTE, Zhang L, Tong YW, et al., 2021. Syntrophic interactions in anaerobic digestion: how biochar properties affect them? Sustainable Environment. 7, 1945282
- Kamagta, 2015. Syntrophy in Anaerobic Digestion. Anaerobic Biotechnology (Environmental Protection and Resource Recovery). Syntrophy in Anaerobic Digestion. 13–30.
- Kleinsteuber, 2018. Metagenomics of Methanogenic Communities in Anaerobic Digesters. Biogenesis of Hydrocarbons, 1-23.
- Kleyböcker A, Liebrich M, Verstraete W, Kraume M, Würdemann H. 2012. Early warning indicators for process failure due to organic overloading by rapeseed oil in one-stage continuously stirred tank reactor, sewage sludge and waste digesters. Bioresource Technol. 123, 534–541.
- Khelaifia S, Raoult D, Drancourt M. 2013. A Versatile Medium for Cultivating Methanogenic Archaea. PLoS ONE 8:e61563.
- Kletzin A. 2007. Metabolism of inorganic sulfur compounds in archaea. In Archaea: Evolution, Physiology, and Molecular Biology. Garrett, R.A., and Klenk, H.-P. (eds). Oxford, UK: Blackwell, pp. 261–274.
- Kong X, Wei Y, Xu S, Liu J, Li H, Liu Y, Yu S. 2016. Inhibiting excessive acidification using zero-valent iron in anaerobic digestion of food waste at high organic load rates. Bioresour Technol. 211, 65-71.
- Kounosu A, Murase K, Yoshida A, Maruyama H, Kikuchi T. 2019. Improved 18S and 28S rDNA primer sets for NGS-based parasite detection. Sc Rep. 9:15789.
- Kurr M, Huber R, König H, Jannasch HW, Fricke H, et al., 1991. *Methanopyrus kandleri*, gen. and sp. nov. represents a novel group of hyperthermophilic methanogens, growing at 110 C. Arch Microbiol. 156, 239–247.
- Kurth JM, Ob de Camp HJM. Welte CU. 2020. Several ways one goal—methanogenesis from unconventional substrates. Applied Microbiol Biotechnol.104, 6839–6854.
- Lang K, Schuldes J, Klingl A, Poehlein A, Daniel R, Brune A. 2015. New mode of energy metabolism in the seventh order of methanogens as revealed by comparative genome analysis of '*Candidatus Methanoplasma termitum*'. Appl Environ Microbiol 81, 1338–1352.
- Laso-Pérez R, Wegener G, Knittel K, Widdel F, Harding KJ, et al. 2016. Termophilic archaea activate butane via alkyl-coenzyme M formation. Nature. 539, 396–401.
- Lara E, Moreira D, Lopez-Garcia P. 2009. The Environmental Clade LKM11 and Rozella Form the Deepest Branching Clade of Fungi. Protist. 161, 116-21.
- Lazarus K, James TY. 2015. Surveying the biodiversity of the Cryptomycota using a targeted PCR approach. Fungal Ecol. 14, 62-70.
- Li Q, Li L, Rejtar T, Lessner DJ, Karger BL, Ferry JG. 2006. Electron transport in the pathway of acetate conversion to methane in the marine archaeon *Methanosarcina acetivorans*. J Bacteriol. 88, 702–710.
- Li Y, Li L, Sun Y, Yuan Z. 2018. Bioaugmentation strategy for enhancing anaerobic digestion of high C/N ratio feedstock with methanogenic enrichment culture. Bioresour Technol. 261, 188–195.
- Li Y, Hou Z, Shi Q, Yanfen C, Zhu W. 2020. Methane Production From Different Parts of Corn Stover via a Simple Co-culture of an Anaerobic Fungus and Methanogen. Front Bioeng Biotechnol. 8:314.
- Li Y, Meng Z, Xu Y, Shi Q, Ma Y, Aung M, Cheng Y, Zhu W. 2021. Interactions between Anaerobic Fungi and Methanogens in the Rumen and Their Biotechnological Potential in Biogas Production from Lignocellulosic Materials. Microorganisms. 9. 190.
- Lier et al., 2008. Biological Wastewater Treatment: Principles, Modelling and Design. IWA Publishing. 415-457.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Lim JW, Park T, Tong YW, Zhongtang Y. 2020. The microbiome driving anaerobic digestion and microbial analysis. Advances in Bioenergy. 5, 1–61.
- Liu Y, Whitman WB. 2008. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. Ann N Y Acad Sci. 1125, 171–189.
- Liu, Y. 2010. Methanopyrales. In "Handbook of Hydrocarbon and Lipid Microbiology. Part 7," (K. N. Timmis, ed.), pp. 605–607. Springer-Verlag, Berlin Heidelberg.
- Liu Y, Beer LL, Whitman WB. 2012. Sulfur metabolism in archaea reveals novel processes. Environment Microbiol. 14, 2632-2644.
- Liu D, Nishida M, Takahashi T, Asakawa S, 2018. Transcription of mcrA Gene Decreases Upon Prolonged Non-flooding Period in a Methanogenic Archaeal Community of a Paddy-Upland Rotational Field Soil. Microb Ecol. 75, 751–760.
- Long F, Wang L, Lupa B, Whitman WB. 2017. A Flexible System for Cultivation of *Methanococcus* and Other Formate-Utilizing Methanogens. Archaea. e7046026.
- Matsubayashi M, Shimada Y, Li YY, Harada H, Kubota K. 2017. Phylogenetic diversity and in situ detection of eukaryotes in anaerobic sludge digesters. Plos One. 12: e0172888.
- Mutungwazi A, Ijoma GN, Matambo TS. 2021. The significance of microbial community functions and symbiosis in enhancing methane production during anaerobic digestion: a review. Symbiosis, 83, 1–24.
- Müller B, Sun L, Westerholm M, Schnürer A. 2016. Bacterial community composition and fhs profiles of low- and high-ammonia biogas digesters reveal novel syntrophic acetate-oxidising bacteria. Biotechnol Biofuels. 9:48.
- Nguyen LN, Nguyen AQ, Nghiem LD. 2018. Microbial Community in Anaerobic Digestion System: Progression in Microbial Ecology. Water and Wastewater Treatment Technologies. 331-355.
- Nielsen HB, Angelidaki I. 2008. Strategies for optimizing recovery of the biogas process following ammonia inhibition. Bioresour Technol. 99, 7995–8001.
- Nzila A, Razzak SA, Zhu J. 2016. Bioaugmentation: An Emerging Strategy of Industrial Wastewater Treatment for Reuse and Discharge. Int J Environ Res, 13, 846.
- Offre P, Spang A, Schleper C. 2013. Archaea in biogeochemical cycles. Annu Rev Microbiol. 67, 437–457.
- Orphan VJ, House CH, Hinrichs KU, McKeegan KD, DeLong EF. 2001. Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. Science. 293, 484–487.
- Poszytek K, Pyzik A, Sobczak A, Lipinski L, Sklodowska A, Drewniak L. 2017. The effect of the source of microorganisms on adaptation of hydrolytic consortia dedicated to anaerobic digestion of maize silage. Anaerobe. 46, 46-55.
- Prabhakaran P, Bhasi Y, Ali S, Narayanan N, Balakrishnan MV, Bhaskaran K. 2016. Community dynamics and significance of anaerobic protozoa during biomethanation of lignocellulosic waste. Renew Energ. 98, 148-152.
- Priya M, Haridas A, Manilal VB. 2008a. Anaerobic protozoa and their growth in biomethanation systems. Biodegradation. 19, 179–185.
- Priya M, Haridas A, Manilal VB. 2008b. Involvement of protozoa in anaerobic wastewater treatment process. Water research. 41, 4639-45.
- Procházka J, Mrázek J, Štrosová L, Fliegerová K, Zábranská J, Dohanyos M. 2012. Enhanced biogas yield from energy crops with rumen anaerobic fungi. Engineering in Life Sciences. 12, 343-351.
- Qin S, Wainaina S, Awasthi SK, Mahboubi A Liu T, et al. 2021. Fungal dynamics during anaerobic digestion of sewage sludge combined with food waste at high organic loading rates in immersed membrane bioreactors. Bioresour Technol. 335:125296.

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- Romero-Güiza MS, Vila J, Mata-Alvarez J, Chimenos JM, Astals S. 2016. The role of additives on anaerobic digestion: A review. Renew Sustain Energy Rev. 58, 1486–1499.
- Saha S, Basak B, Hwang JH, Salama ES, Chatterjee PK, Jeon BH. 2020. Microbial Symbiosis: A Network towards Biomethanation. Trends in Microbiology, 28, 968-984.
- Saengkerdsub S, Ricke S. 2013. Ecology and characteristics of methanogenic archaea in animals and humans. Crit Rev Microbiol. Early Online: 1–20.
- Schleper C, Nicol GW. 2010. Ammonia-oxidising archaea - physiology, ecology and evolution. Adv Microb Physiol. 57, 1–41.
- Schink B, Stams AJM. 2013. Syntrophism among Prokaryotes. In: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, KH., Stackebrandt, E. (eds.). The Prokaryotes. Springer, New York, NY. https://doi.org/10.1007/0-387-30742-7_11.
- Schlegel K, Müller V. 2011. Chapter twelve – Sodium Ion Translocation and ATP-Synthesis in Methanogens. In: Rosenzweig AC, Ragsdale SW (eds.). Methods in Methane Metabolism, Part A. 494, 233 – 255.
- Singh A, Singh RS, Upadhyay SN, Joshi CG, Tripathi AK, et al. 2012. Community structure of methanogenic archaea and methane production associated with compost-treated tropical rice-field soil. FEMS Microbiol Ecol. 82, 118-134
- Smith KS, Ingram-Smith C. 2007. *Methanosaeta*, the forgotten methanogen? Trends Microbiol. 15:150-155.
- Sowers KR. 2019. Methanogenesis. In: Thomas M. Schmidt (eds.). Encyclopedia of Microbiology (Fourth Edition), Academic Press, 2019, 53-73, ISBN 9780128117378.
- Steinberg LM, Regan JM. 2009. mcrA-targeted real-time quantitative PCR method to examine methanogen communities. Appl Environ Microbiol. 75, 4435–4442.
- Steinhaus B, Garcia ML, Shen AQ, Angenent LT. 2007. A Portable Anaerobic Microbioreactor Reveals Optimum Growth Conditions for the Methanogen *Methanosaeta concilii*. Appl Environ Microbiol. 73, 1653–1658.
- Stiles ME, Holzapfel WH. 1997. Lactic acid bacteria of foods and their current taxonomy. Int J Food Microbiol. 36, 1–29.
- Summers ZM, Fogarty HE, Leang C, Franks AE, Malvankar NS, Lovley D. 2010. Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. Science. 330, 1413–1415.
- Suryawanshi PC, Chaudhari AB, Kothari RM. 2010. Mesophilic anaerobic digestion: first option for waste treatment in tropical regions. Crit Rev Biotechnol. 30, 259–282.
- Thauer RK, Jungermann K, Decker K. 1997. Energy conservation in chemotrophic anaerobic bacteria. Microbiol Mol Biol Rev. 41, 100–80.
- Thauer RK, Kaster AK, Seedorf H, Buckel W, Reiner H. 2008. Methanogenic archaea: ecologically relevant differences in energy conservation. Nat Rev Microbiol. 6, 579–591.
- Tian H, Yan M, Treu L, Angelidaki I, Fotidis I. 2019. Hydrogenotrophic methanogens are the key for a successful bioaugmentation to alleviate ammonia inhibition in thermophilic anaerobic digesters. Bioresour Technol. 293, 122070.
- Timonen S, Bomberg M. 2009. Archaea in dry soil environments. Phytochem Rev. 8, 505–518.
- Wang H, Fotidis IA, Angelidaki I. 2015. Ammonia effect on hydrogenotrophic methanogens and syntrophic acetate-oxidizing bacteria. FEMS Microbiol Ecol. 91. fiv130.
- Welte C, Deppenmeier U. 2014. Bioenergetics and anaerobic respiratory chains of aceticlastic methanogens. Biochim Biophys Acta. 1837, 1130–1147.
- Wen X, Yang S, Horn F, Winkel M, Wagner D, Liebner S. 2017. Global Biogeographic Analysis of Methanogenic Archaea Identifies Community-Shaping Environmental Factors of Natural Environments. Front Microbiol. 8:1339.
- Wolfe RS. 2011. Techniques for cultivating methanogens. Methods Enzymol. 494, 1-22.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Wolfe RS, Metcalf WW. 2010. A vacuum-vortex technique for preparation of anoxic solutions or liquid culture media in small volumes for cultivating methanogens or other strict anaerobes. Anaerobe. 16, 216–219.
- Worm P, Müller N, Plugge CM, Stams AJM, Schink B. 2010. Syntrophy in methanogenic degradation. In: Hackstein J (ed.) (Endo)symbiotic methanogenic archaea. Microbiology monographs, vol 19. Springer, Berlin, Heidelberg, pp 143–173.
- Van Steendam C, Smets I, Skerlos S, Raskin L. 2019. Improving anaerobic digestion via direct interspecies electron transfer requires development of suitable characterization methods. Curr Opin Biotechnol. 57, 183–190.
- Venkiteshwaran K, Bocher B, Maki J, Zitomer D. 2016. Relating Anaerobic Digestion Microbial Community and Process Function: Supplementary Issue: Water Microbiology. Microbiol Insights. 8 (S2).
- Vinzelj J, Joshi A, Insam H, Podmirseg SM. 2019. Employing anaerobic fungi in biogas production: challenges & opportunities. Bioresour Technol. 122687.
- Wilken SE, Seppälä S, Lankiewicz TS, Saxena M, Henske JK, et al. 2019. Genomic and proteomic biases inform metabolic engineering strategies for anaerobic fungi. Metabolic Engineering Communications. 10:e00107.
- Wrede C, Dreier A, Kokoschka S, Hoppert M. 2012. Archaea in Symbioses. Archaea, 1, 596846.
- Yang Z, Wang W, Liu C, Zhang R, Liu G. 2019. Mitigation of ammonia inhibition through bioaugmentation with different microorganisms during anaerobic digestion: Selection of strains and reactor performance evaluation. Water Research. 155, 214-224.
- Yan Y, Yan M, Ravenni G, Angelidaki I, Fu D, Fotidis I. 2022. Novel bioaugmentation strategy boosted with biochar to alleviate ammonia toxicity in continuous biomethanation. Bioresource Technology, 343, 126146.
- Yamada T, Sekiguchi Y, Hanada S, Imachi H, Ohashi A, Harada H, Kamagata Y. 2006. *Anaerolinea thermolimosa* sp. nov., *Levilinea saccharolytica* gen. nov., sp. nov. *and Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and description of the new classes Anaerolineae classis nov. and Caldilineae classis nov. in the bacterial phylum Chloroflexi. Int J Syst Evol Microbiol. 56, 1331–1340.

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6. Manipulability of anaerobic microbiomes

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6.1 The AD microbiome

The term Microbiome was introduced by Whipps et al. in 1988. The word is a combination of the terms “Micro” and “Biome”, which is related to a characteristic microbial population in a defined habitat. We find microbiomes everywhere around us. In the soil, in the air, in water and in countless sub-niches. The AD-microbiome is due to the high number of microbial species in combination with its technological relevance and its potential for a biobased industry a great playground for microbiome researchers all over the world. A recent review by Berg *et al.* (2008) mentions the money that is spend on microbiome research, stating that US$1.7 billion were spent during the last decade in the medical research. Microbiome associated research activity is less intense in the field of agronomy for now. However, Berg *et al.* highlights that “Agricultural products based on the microbiota are one of the fastest growing sectors “. In this relation, a tremendous effort is made to understand the microbiomes that are involved in AD-processes. A simplified overview of the structure of anaerobic microbiomes can be found in the works of Sundberg *et al.* (2013) and Abendroth *et al.* (2015), in which microbiomes from sewage sludge digestion and co-digestion are distinguished. Additionally, Abendroth *et al.* highlights differences between CSTR systems and leach-bed systems. Both authors distinguish between bacterial and archaeal key-players. In simple terms, it can be said that bacteria are responsible for the first 3 AD phases (hydrolysis, acidogenesis, and acetogenesis). Methanogenic archaea are key players in the last phase (methanogenesis). Bacteria hydrolyze complex polymers in the first phase and convert them step by step into organic acids during acidogenesis. Eventually, all organic acids are converted into acetate, which is the main substrate for methanogenesis (Robles *et al.*, 2018). In the first three stages, the following bacterial phyla are abundant: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Chloroflexi, Spirochaetes, Thermotogae and Synergistetes. Below them, particularly Chloroflexi and Proteobacteria are more abundant in sewage sludge digestion than in co-digestion (Sundberg *et al.*, 2013; Abendroth *et al.*, 2015). Spirochaetes are abundant in leachate from leach-bed systems and in sewage sludge digesters as well. In contrast, Spirochaetes are not abundant in CSTRs, very like due to the high percentage of total solids (TS). Usually, CSTRs show very high ratios of Firmicutes and Bacteroidetes. The latter one is abundant ins sewage sludge digestion and leachate from leach-bed digestions as well. Sundberg *et al.* and Abendroth *et al.* describe further that below the genera of methanogenic archaea, particularly *Methanobacterium*, *Methanoculleus*, *Methanospirillum, Methanothrix,* (also known as *Methanosaeta*), *Methanobrevibacter*, *Methanothermobacter*, *Methanomethylovorans* and *Methanosarcina* are abundant. Below them, *Methanothrix, Methanothermobacter* and Methanomethylovorans are especially abundant in sewage sludge digestion. In contrast, *Methanobacterium*, *Methanoculleus*, *Methanosarcina* as well as *Methanobrevibacter* seem to be abundant in co-digesters. Again, Abendroth *et al.* describes differences between

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leachate from leach-bed systems and CSTRs. While *Methanoculleus* is particularly common in CSTRs, *Methanosarcina* dominates the leachate from leach-bed reactors.

6.2 Manipulating the AD-blackbox

Even if a targeted neofunctionalization of microbiomes in biogas plants according to the state of the art has so far hardly been used, there exists an extensive knowledge about optimal nutrition and chemical process parameters. An important parameter for example is the C:N:P:S ratio, which, according to Weiland (2009) is optimal at an ratio of 600:15:5:1. Carbon and nitrogen are the basic elements that build up organic matter and cellular structures. Sulfur is needed for the growth of methanogens and several amino acids. Phosphate is an essential energy carrier (Nsair et al., 2020). Apart from these basic elements, there is a wide range of other nutrients that are important and extensively studied. The review article by Nsair et al. has recently summarized many process parameters such as optimal values for ammonia, sulfides, light and heavy metals (e.g., Calcium, Cobalt, Molybdenium, Nickel, Zinc, Iron and other). Apart from the optimal feedstock composition, it is of high importance to choose the right environmental parameters. The pH is usually kept around 7 and especially low pH-values are inhibiting for the involved methanogenic archaea. Moreover, it was found that there is a correlation between pH, undissociated volatile fatty acids (VFAs) and process inhibition. Especially undissociated VFAs have an inhibitory effect. At lower pH-values a higher ratio of a given VFA is protonated. Hence, a given acid concentration can cause varying pH-dependent inhibition (Kroiss and Svardal, 2005). To prevent a low pH-value, the organic loading rate needs to be chosen carefully and different loading rates need to be applied for different substrates. To give here an example: Agricultural waste is, according to Nasir et al. (2020) slower degraded as food waste, as it contains higher amounts of lignocellulose. In turn, VFAs are faster accumulated during the degradation of food waste, which in turn can result in a pH-drop. A low pH increases the concentration of protonated VFAs and inhibits eventually the process (Kroiss and Svardal, 2005). When nitrogen enriched substrates, such as chicken manure, are applied, high ammonia concentrations prevent low pH-values, which allows methane formation even with unusual high substrate concentrations (Abendroth et al., 2015). Hence, the sensitivity of AD plants to VFAs also depends on the buffer capacity. Although ammonia can stabilize the pH, this does not mean that methanation at high level of ammonia occurs effective. In fact, ammonia is a known inhibitor for anaerobic digestion. It can pass the membrane of several microorganisms (e.g., *Methanobacterium formicicum* or *Methanospirillum hungatei*), which in turn leads to a loss of cytoplasmic K^+ ions (Yenigün and Demirel, 2013). Another substance that is important for the buffer capacity is bicarbonate. Even if high concentrations of biocarbonate can have inhibiting effects as well (Lin et al., 2013), bicarbonate buffer systems are generally acknowledged as important for the AD process (Kasali et al., 1989; Lin et al., 2013). The bicarbonate buffer system can be created just from process water and carbon dioxide, which is formed during the fermentation. In fact, it’s the same buffer system, as in our blood, which is, however, strongly affected by pH. A pH < 7,35 or > 7,45 can already impair its buffering capacity (Rhoades and Bell, 2012). A low pH shifts the equilibrium of the buffer system in the direction of the CO_2, so that the buffer is driven out. As low pH-values impair the puffer system and result in higher NH_3-

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levels as well as protonated (inhibiting) VFAs, sharp drops in the pH value should be avoided as far as possible. Because of this, the German biogas industry elaborated a new parameter, the so-called FOS/TAC. It describes the ratio of organic acids (FOS) to the inorganic buffer capacity (TAC). The FOS/TAC can now also be found in international research articles, so that this parameter is developing into an internationally recognized process parameter (Lili et al., 2011; Costa et al., 2016). Finally, it needs to be highlighted, that the ratio of dissociated and undissociated substances is also affected by temperature. The literature describes AD-processes at thermophilic-, mesophilic-, and as well psychrophilic temperatures (Kroiss and Svarland, 2005). Psychrophilic temperatures are particularly interesting in respect to high ammonia levels. As lower temperatures can shift the equilibrium from ammonia to the less toxic ammonium, literature discusses the suitability of anaerobic digestion at low temperatures to alleviate the toxic effect of ammonia. The positive effect of lower ammonia levels at lower temperatures is counteracted by the fact that the lower temperature also lowers the rate constant for methanogenesis (Garcia and Angenent, 2009). On the other hand, one can conclude that especially thermophilic AD-plants might be sensitive to ammonia-stress. The approaches to optimizing fermentation processes described here only scratch the surface of what is known about the influence of chemical parameters on the process. This chapter could probably be expanded into an entire book. Nevertheless, this chapter clearly indicates the immense knowledge about adaptation strategies that help to set high-performance processes in motion, even if the underlying microbiome is treated as a black box.

6.3 Microbiome engineering

It is a tempting idea to modify complex microbiomes to achieve a specific function. AD microbiomes could become faster and more robust, the risk of germs could be reduced, or completely new valuable substances could be produced instead of methane. The goal of high-value products from anaerobic digestion is partially in reach, even without fully resolving the AD-black box. This can be explained as follows: If particularly high-volume loads with easily digestible substrates are used, low pH values can be achieved, which inhibit methanogenic archaea. It is thus possible to carry out a separate hydrolysis/acidification without the accumulated metabolites being broken down by methanogenesis. Such an approach allows the enrichment of medium-length carboxylic acids (De Groof et al., 2019) and hydrogen (Wang and Yin, 2018). Multiple articles have already demonstrated the possibility of combining dark fermentation with subsequent methanation (Abendroth *et al.*, 2017, Wang *et al.*, 2018, Wainaina *et al.*, 2019). If further fermentation- and pretreatment steps are applied, it is even possible to enrich valuable fatty acids such as caproic acid (Chen et al., 2017).
It is further possible to combine methanogenic stages with pure culture approaches. An example is the production of succinic acid. It has been demonstrated that biogas can be purged with the help of the bacterium *Actinobacillus succinogenes*. If biogas is injected into a separate fermentation stage, *A. succinogenes* can capture CO_2. The fixed CO_2 is then co-fermented with sugar, which improves the efficiency of *A. succinogenes* in producing succinic acid (Gunnarsson *et al.*, 2014).

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Apart from the two-stage approach, which separates methanogenesis from the phases of biogas formation, researchers are also interested in the question, weather the methanogenic stage itself could be optimized. Even if there are only few articles on this so far, some authors have tried to add powerful microorganisms directly to the methane stage (bioaugmentation). To give here few examples: Mulat *et al.* (2018) used the strain *Caldicellulosiruptor bescii* to improve methane production from lignocellulosic biomass. Montusiewicz *et al.* (2020) used a microbial consortium from Yellowstone National Park (USA), which tends to an intracellular accumulation of heavy-metal ions to manipulate heavy metal concentrations. Mutschlechner *et al.* (2020) used a soil derived inoculum, which improved the methane production and counteracted common process failures.
Despite that there are some promising examples in respect to bioaugmentation, it remains questionable, weather a long-lasting affect can be achieved on industrial scale. For microorganisms it might be difficult to assert themselves in an alien environment against organisms that have already adapted to the domestic biome. In this relation, a recent article about bioaugmentation states that "*At present it is still unclear which factors determine the success of such a treatment*" (Müller and Mahro, 2001). To make bioaugmentation a reliable method, new approaches and strategies are required. Thompson *et al.* (2005) highlight in their work that modern approaches in molecular biology and analytical chemistry allow a more targeted search for microorganisms that are suited for bioaugmentation. According to Thompson *et al.* is this "*information-based approach*" already more efficient than the former "*black-box*" approaches. And modern methods of molecular biology indeed had a major impact on the understanding of AD microbiomes. Since slightly more than a decade, next generation sequencing is applied, beginning with the Roche 454 sequencing technology in 2005 (Selzer et al., 2018). This ushered in a flood of research articles trying to break down the black box of anaerobic microbiomes piece by piece. This major advancement is also highlighted by a recent article from Laczi *et al.*, (2020) entitled "*New Frontiers of Anaerobic Hydrocarbon Biodegradation in the Multi-Omics Era*", where OMICs is a suffix that denotes the analysis of a population of elements with similar characteristics. Recent literature describes the application of metagenomics (e.g., Bertucci *et al.*, 2019; Becker *et al.*, 2020), proteomics (e.g., Bargiela *et al.*, 2015; Zhang *et al.*, 2015), transcriptomics (Maus et al., 2016; Gruninger et al., 2018) or metabolomics (e.g., Bargiela *et al.*, 2015; Artegoitia *et al.*, 2017).

As knowledge about taxonomic profiles of anaerobic microbiomes progresses, so does functional understanding. An important recent finding is the ability of several microorganisms to directly exchange electrons between each other. Since 2005 first research articles appeared, which indicated the existence of microbial nanowires that can transport electrons (Reguera *et al.*, 2005; Gorby *et al.*, 2006; Reguera *et al.*, 2006). The discovery that by using conductive particles DIET is also possible without microbial nanowires, spread a gold rush mood for agents that can be used to stimulate direct interspecies Electron Transfer (DIET). Many of the tested materials are highlighted in a recent review by Martins *et al.* (2018). A particularly exotic article by Beckmann et al. (2016) describes the synthesis of novel phenazine crystals, which are stored between microorganisms and there promote the direct exchange of electrons. The promising prospects for the use of DIET are based on an integrated model with syntrophic reaction communities. The review article from Schink and Stams (2006) gives a comprehensive insight into syntrophic relations within anaerobic microbiomes. It describes

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that the degradation of several metabolites, such as acetic acid, propionic acid, butyric acid, or other metabolites occurs under thermodynamically unfavorable conditions, with very little energy gain for the involved microorganisms. From a thermodynamic point of view, the reverse reaction that consumes hydrogen is preferred. However, if hydrogen is actively removed from the system, the hydrogen consuming reverse reaction can be inhibited. In syntrophic communities, so-called hydrogenotrophic methanogens form methane from hydrogen and carbon dioxide, which ensures low levels of hydrogen. In turn, this allows the degradation of metabolites, such as acetic-, propionic-, or butyric acid. The hydrogen that is generated when these acids break down serves as a mediator for electrons. With the help of microbial nanowires, the electrons to be given off can be passed on to hydrogenotrophic methanogens without releasing hydrogen. This in turn, facilitates the degradation of the respective VFAs (Xu *et al.*, 2018, Zhang *et al.*, 2019). The concept of DIET also explains positive effects that are observed when electricity is used for bio stimulation. In 2005 the concept of so-called microbial electrolysis cells (MECs) has been introduced by two institutions in parallel (Liu et al., 2005; Rozendal and Buisman, 2005). The idea behind MECs is that the breakdown of organic substances leads to an excess of electrons, which can be transferred to an anode. With a low starting voltage (0.2-0.8V), these electrons can be conducted to a cathode. The degradation processes also lead to the release of protons, which diffuse towards the cathode. A catalytic surface (usually platinum) allows electrons to be transferred to the protons to generate hydrogen (Kadier *et al.*, 2016). Although the idea was originally developed for the synthesis of hydrogen, it is now known that the microbial methane formation can also be stimulated with the help of electrodes (Yanuka-Golub *et al.*, 2019).

6.4 Combined application of Methanosarcina and DIET

6.4.1 General remarks on *Methanosarcina*

Current research suggests that *Methanosarcina* may be an efficient high-performance methanogen. In the present thesis several publications address the enrichment of the genus *Methanosarcina* (Hardegen *et al.*, 2018; Abendroth *et al.*, 2020; Heitkamp *et al.*, 2021), which is why a separate subchapter is dedicated to this genus at this point. The methanogen *Methanosarcina* is a genus of methanogenic archaea belonging to the phylum Euryarchaeota (NCBI: txid2207). While mainly bacteria play a role in the acidification stages (as discussed in the previous chapter), methanogenic archaea are the key players in methanogenic stages. Currently, three metabolic pathways for methane formation are known (KEGG, map0068), whereby methane from H_2 / CO_2 (hydrogenotrophic pathway), acetate (acetoclastic pathway) or methanol / methylamines / methyl sulfide (methylotrophic pathway) is produced (Vanwonterghem *et al.*, 2016). Methanogens, which are described as particularly common and dominant in biogas plants, belong to the genera *Methanoculleus*, *Methanosarcina*, *Methanobrevibacter*, *Methanobacterium*, *Methanothermobacter* and *Methanosaeta* (Abendroth *et al.*, 2015; Sundberg *et al.*, 2013), whereby it should be mentioned here that *Methanosaeta* was renamed *Methanothrix* some time ago (Garrity *et al.*, 2011). Even if the literature already provides a lot of information on methanogenic archaea, the causes that

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lead to the accumulation of certaine methane-forming archaea are not sufficiently investigated. The genus *Methanoarcina* has been of particular interest for some time, as some species of this genus dominate all metabolic routes of methane production (Buan et al., 2011). *Methanosarcines* form very characteristic clumps, which allow a good differentiation from other methane producers. They are often described as non-motile (e.g., Shimizu *et al.*, 2011; Kern *et al.*, 2016; Oshurkova *et al.*, 2020), although it was suggested that they can move due to gas filled vesicles (Meader *et al.*, 2006). In addition, there are indications in the literature that species of the genus *Methanosarcina* are high-performance organisms which could be of particular interest to the industry.

6.4.2 Industrial relevance of *Methanosarcina*

Methanosarcina is a genus that can be found in many biogas plants. Above all, they are found in high-performance fermenters, which use co-substrates to achieve a high concentration of chemical oxygen demand (COD) and organic acids (Abendroth *et al.*, 2015). In contrast, the genus *Methanothrix* (formerly *Methanosaeta*), is mainly found in digestion towers of sewage treatment plants (Sundberg *et al.*, 2013; Abendroth *et al.*, 2015). The activated sludge used as a substrate can be classified as low-energetic compared to substrates such as corn silage or sugar beet silage. This is due to the fact that over 90% of the COD is broken down in the activated sludge process (Fang *et al.*, 1993). A simple explanation for the dominance of the genus *Methanothrix* in digestion towers is that it is able to absorb acetic acid more efficiently, as is the case with the genus *Methanosarcina* (De Vrieze, 2014). This can be explained very well on the biochemical level. While *Methanothrix* metabolizes acetate efficiently at a concentration of K_S = 0.5 mM, this value is between 3 - 5 for *Methanosarcina*. *Methanosarcina* thus absorbs acetate much more inefficiently but metabolizes it more efficiently. The reason for this lies in the different activation mechanisms for acetate. *Methanthrix* uses the acetyl-CoA synthase for this, while *Methanosarcina* uses the acetate kinase. The maximum speed for the acetate kinase of *Methanosarcina thermophila* is 660 µmol mg^{-1} min^{-1} and the acetyl-CoA synthase of *Methanothrix soehngenii* reaches 55 µmol mg^{-1} min^{-1} (Jetten *et al.*, 1991). *Methanosarcina* is therefore about 12 times faster than *Methanothrix*.
As early as 2012, in a review article by De Vrieze *et al.* under the title "*Methanosarcina: The rediscovered methanogen for heavy duty biomethanation*", the genus *Methanosarcina* was highlighted as a high-performance organism for biogas plants. Key aspects to be mentioned in this context are robust behavior at high concentrations of salt and ammonium. It was further emphasized that *Methanosarcines* are active in a broad temperature range and that, as already mentioned above, they show a robust behavior with high loading rates. Since then, several other beneficial characteristics have been described for the genus *Methanosarcina*. These include, for example, the ability to use diverse substrate sources (Lackner *et al.*, 2018) and the degradation of some toxic substances, such as tetramethylammonium (Chen et al., 2017), phenols (Poirier et al., 2018). Moreover, *Methanosarcina* shows a high robustness against nanoparticles made of silver oxide, titanium oxide or zinc oxide (Eduok *et al.*, 2017). Another special ability of *Methanosarcina* is the direct interspecies electron

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transfer (DIET), which is often described for genera *Methanosarcina* and *Methanothrix* (Martins *et al.*, 2018). Since the genus *Methanothrix* is more likely to be found in methane reactors with low levels of COD and organic acids, the industrial relevance of DIET in connection with the genus *Methanosarcina* should be pointed out at this point.

6.4.3 Increasing the rate of direct interspecies electron transfer (DIET)

Interspecies electron transfer is the prerequisite for methanogenesis. The H_2 interspecies transfer (HIT), in which hydrogen is used as an electron carrier, has been particularly well investigated (Sieber *et al.*, 2012). An alternative to HIT is direct electron transfer between species (DIET). For a direct transfer of electrons between bacteria and *Methanosaeta (Methanotrix) harundinacea*, cellular outlets with metal-like conduction properties for electrons are necessary (Rotaru *et al.*, 2014). In contrast to *M. harundinacea*, a DIET has been described several times for the genus *Methanosarcina*, which is based on the use of inorganic, extracellular metal surfaces (Kato *et al.*, 2012; Liu *et al.*, 2012). An example is conversion of ethanol to acetate with the aid of ferrihydrite, as described by Tang *et al.* (2016). In the respective work it was observed that depending on whether phosphate is added or not, the addition of ferrihydrite leads to the formation of magnetite or vivianite. During the degradation of ethanol to acetate by *Geobacter* there is an excess of electrons. Using magnetite or vivianite, *Geobacter* can conduct the surplus electrons via magnetite or vivianite to *Methanosarcina*, which is using the electron for the reduction of CO_2.

The DIET and the ability of *Methanosarcina* to interact with extracellular, metallic, non-biological surfaces offer interesting application possibilities in industry, which has already been introduced in the introduction of this thesis. Kato & Igarashi (2018) described an acceleration of methanogenesis in methane reactors by adding iron oxide and sulfate. A similar observation was made by Ye *et al.* (2018), where the addition of 20 g L^{-1} red mud ("red mud") accelerated methane accumulation by 35.5%. At this point it should be emphasized that Ye *et al.* also observed a positive effect on hydrolysis & acidification.
However, the ability of *Methanosarcina* to DIET is not only relevant for the biogas industry. There are authors who highlight *Methanosarcina* in connection with biological remediation. For example, Holmes *et al.* (2018) described that *Methanosarcina* has a high potential to contribute to uranium reduction in uranium-contaminated soils when acetate is added (Acetate-Promoted Groundwater Bioremediation). In connection with the interaction between *Methanosarcina* and metallic surfaces, *Methanosarcina* is therefore also described as being an indicator organism for contamination (Pei *et al.*, 2018).

In a biogas plant, the microbial diversity is very high. About 300 different species can explain 80% of the process biocenosis of a biogas reactor (Kirkegaard *et al.*, 2017). This high diversity of microorganisms forms a robust microbiome, which makes it difficult to accumulate a certain species on purpose (Abendroth, 2018). Finding influencing parameters for the enrichment of the genus *Methanosarcina* is therefore a great challenge. In this context, it is helpful to know that different types of sludge from digestion plants naturally have a different profile of methanogenic archaea (Abendroth *et al.*, 2015). The fact that digested sewage sludge

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accumulates species of the genus *Methanothrix* can be explained by the fact that *Methanothrix* is better suited to the uptake of acetic acid in low concentration ranges than is the case with the other genera. A value of 3000 mg acetic acid was given as the limit value (De Vrieze, 2014). Above this acetic acid concentration, the high-performance methane-forming agents *Methanoculleus* and *Methanosarcina* occur more intensely, whereby the genus *Methanosarcina* is particularly interesting due to its ability for direct interspecies electron transfer. However, as viscous sludge tends to accumulate the genus *Methanoculleus* (for which DIET was not described so far) this raises the question how a *Methanosarcina* prevalence can be achieved with high acid loads?

A hint can be found in the publication by Abendroth *et al.* (2015): In contrast to thick sludge from stirred tank systems, the dry matter content (TS) in the leachate from leach-bed digesters is significantly reduced. In recent work by Hardegen *et al.*, 2018, there is an indication that the dry matter content has an influence on the profile of methanogenic archaea. A low percentage of total solids seems to favor the accumulation of the genus *Methanosarcina*. Hardegen *et al.* increased gradually the chemical oxygen demand (COD) in digested sewage sludge by adding liquid and solid co-substrates. In line with the expected value, there was a population shift. Digested sewage sludge charged with liquid biomass showed an increase in *Methanosarcina*. According to the work by Abendroth *et al.* (2015), one could have expected an enrichment of the genus *Methanoculleus* in the experiments fed width solids by Hardegen *et al.* This expected population "shift" did not materialize, which might be explained by an accumulation of inhibiting substances. Since the here presented experiments by Hardegen *et al.* did not reache a "steady state", the continuously changing conditions could also have caused a stressful situation that favored the accumulation of the genus *Methanosarcina.* As already described above, the genus *Methanosarcina* is efficient compared to other methane producers in dealing with stressful conditions. This statement is also in line with a recent publication by Town and Dumonceaux (2016). Reactors that got into acidosis, were reactivated with an inoculum, with an increase in the genus *Methanosarcina* being observed.
A first application for the observations made by Hardegen *et al.* could be given in a slowly increasing loading rate in sewage sludge digesters in combination with the use of conductive particles. The loading rate might be increased by switching from mono-digestion do co-digestion using additional co-substrates, which was also the case in Hardegen *et al.* In relation to the application of DIET, however, it would still have to be demonstrated that the processes described by Hardegen *et al.* results in a prevalence of the genus *Methanosarcina* that persists over a longer period. Since the interspecies electron transfer has been described for both the genera *Methanosaeta* (*Methanothrix*) and *Methanosarcina* (Rotaru *et al.*, 2014), its use by adding electrically conductive particles is conceivable, regardless of whether additional biogenic co-ferments are used in addition to the sewage sludge, albeit the process would be much more efficient, if *Methanosarcina* would be enriched. A disadvantage of a targeted enrichment of *Methanosarcina* would be that there would have to be a continuously high concentration of organic acids. This contradicts the goal of maximum degradation and would require subsequent digestion stages in order to minimize the C-load. A solution here could be a baffled reactor (ABF). ABF reactors a separated in several baffles. In the beginning a high loading rate can be applied, which is decreasing continuously. The system is

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known to be resistant to organic shock loading, show improved biomass retention and lower sludge yields (Barber and Stuckey, 1999).

Apart from sewage sludge digestion (water treatment) it would also be interesting to refurbish existing high-performance biogas plants (typical co-digesters that ferment agricultural residues, food waste or municipal waste). One hurdle here is the typically high concentration of cells of the genus *Methanoculleus*. An exception is the leach-bed digestion, as leachate is typically enriched in *Methanosarcina*. However, systems with highly viscous sludge (continuous stirred tank reactors of plug flow fermenters) are favored by the genus *Methanoculleus* (Abendroth *et al.*, 2015). The existing literature so far does not describe any approaches to suppress *Methanoculleus* in favor of the genus *Methanosarcina* in such systems. With regard to a targeted application of DIET, however, it would make sense to achieve a prevalence of the genus *Methanosarcina* in solids enriched reactors, fixed beds or biofilters. One way to achieve this is the application of very harsh conditions. As already described above, *Methanosarcina* shows a robust behavior against low pH-values, high concentrations of COD, salt, and ammonia. A possible experimental approach here would be the use of stressful situations (e.g., temperature shocks, cyclical changes in nutrient conditions or cyclical fluctuations in pH values), which are carried out directly in fixed beds, solid-rich reactors, fixed beds or biofilters. Particularly high ammonium content could be of interest here, since Dai *et al.* (2016) described an enrichment of the genus *Methanosarcina* in a laboratory experiment, using dehydrated TS-rich sewage sludge as a mono-substrate and causing ammonium stress (5,000 mgN L^{-1}). In this relation, the work from Heitkamp *et al.* (Publication 5 of the present thesis) must be highlighted. Heitkamp *et al.* applied biochar on multiple industrial CSTRs. Several of them had high nitrogen concentrations and three of them had even higher concentrations as described by Dai *et al.* (>5,000 mgN L^{-1}). But still, Heitkamp *et al.* described a surprisingly low amount of *Methanosarcina*. An explanation for this observation might be that fact that *Dai et al.* used digested sewage as seed sludge, which is typically enriched with the genus *Methanothrix.* With increasing loading rates, *Methanosarcina* has a selection advantage compared to *Methanothrix*. As CSTR reactors as the ones investigated by Heitkamp *et al.* are typically enriched in *Methanoculleus* (Abendroth *et al.*, 2015), it might be harder for *Methanosarcina* to compete with *Methanoculleus*. It also needs to be highlighted that in contrast to the here presented results by Hardegen *et al.*, the enrichment of *Methanoculleus* was possible in the work by Dai *et al.* despite high concentrations of solids, which is in contrast to the works by Abendroth *et al.* (2015) and Hardegen *et al.* (2018). In summary, it can be said at this point that it becomes clearer which selection pressures influence *Methanosarcina*, albeit this question has not yet been finally clarified. One should also ask oneself whether the combination of particularly harsh conditions (high concentrations of COD, salt, and ammonium) is the right method for enriching the genus *Methanosarcina*, as this could nevertheless reduce the overall efficiency of the system. Therefore, the search for suitable selection pressures to enrich the genus *Methanosarcina* continues. On one hand, the addition of conductive particles itself could already improve the conditions for *Methanosarcina*, which is discussed in the following subsection.

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6.4.4 Light harvesting and electroactive microbiomes

Another influencing factor that seems to favor *Methanosarcina* is light. In the second publication of the present thesis, a red biofilm was metagenomically analyzed. The biofilm developed unexpectedly on the inner wall of a transparent biogas reactor (Abendroth *et al.*, 2020). At first, it was assumed that it could be algae. However, since no DNA amplification of typical eukaryotic gene sequences was possible, the taxonomic profile based on 16S-rRNA gene amplicon high-throughput sequencing showed that the red colour can come from phototrophic bacteria. Typical phototrophic bacteria are sulfur bacteria, purple nonsulfur bacteria, green sulfur bacteria, and green and red filamentous anoxygenic phototrophic bacteria (Frigaard, 2016). In the case of the red biofilm and based on 16S-rRNA gene sequencing, the genus *Rhodopseudomonas* (purple nonsulfur bacteria) in particular was found in high abundance. Metagenomic sequencing indicated later that particularly *Rhodopseudomonas feacalis* was present (Abendroth *et al.*, 2020). Several reports can be found on microalgae in relation to anaerobic digestion (e.g., Ermis *et al.*, 2020; Xia and Murphy, 2016). However, those studies only refer to algae that are grown on digestate that has left already the methanogenic digester. Therefore, the study by Abendroth *et al.* is generally in agreement with the existing literature on the fact that no eucaryotic algae were found. In relation to phototrophic bacteria, one can now ask the question, which positive effects could be brought about by light in biogas reactors. One positive effect could be an oxygen release, as oxygen is a typical product from photosynthesis. In a recent study, the general belief that oxygen is toxic to biogas reactors has been refuted. It was emphasized that a communalization of aerobic and anaerobic microorganisms is possible, and that the oxygen can even have a positive effect on the process efficiency (Botheju and Bakke, 2011). However and in the presented work from Abendroth *et al.* (2020), no biochemical processes have been described that indicate an oxygen release. In fact, a study from 2016 states that none of the known anoxygenic phototrophic bacteria can oxidize water (Hanada, 2016), which makes it very unlikely that oxygen is naturally produced in biogas reactors. One might suspect that aerobic phototrophs could possibly be enriched under microaerophilic conditions. However, it has been shown in the case of known aerobic phototrophs that they require high oxygen concentrations, and that the photosynthesis rate is severely restricted when the amount of oxygen decreases (Yurkov and Beatty, 1998). It is therefore highly questionable whether phototrophic bacteria could cause a release of oxygen. On the other hand, a recent study needs to be mentioned, which highlights the O_2 production by cyanobacterial mats. These mats are not fully understood as they involve a complex metabolic network resulting from a highly diverse microbial community (Dick *et al.*, 2018). In addition to releasing oxygen to naturally induce microaerophilic conditions, cyanobacteria could also contribute to decrease the H_2S concentration. At H_2S concentrations > 200 µM, *Cyanobacteria* show the capability of performing a sulfide-dependent photosynthesis (Cohen *et al.*, 1986). With H_2S having a molar mass of 34,082 g mol^{-1}, 200 µM corresponds to 0.034 mg L^{-1}. In practice, significantly higher H_2S concentrations are conceivable, with process inhibition at 22 mg L^{-1} in thermophilic and 50 mg L^{-1} in mesophilic reactors to be expected (Haghighatafshar *et al.*, 2012). In the case of the here presented work from Abendroth *et al.*, no Cyanobacteria were found in the red biofilm. However, it is known that Cyanobacteria can be present in anaerobic digesters. For example,

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Abendroth *et al.* (2017) described a high abundance of Cyanobacteria in separated acidification stages, although they were not able to differentiate between Cyanobacteria and chloroplasts from biomass. Also, in publication 5 of the present thesis Cyanobacteria were observed. While indeed 0.3% of the observed reads correspond to chloroplasts (PCC-6307), 1.6% of the reads were assigned to the Cyanobacterium *Cyanobium* (Heitkamp *et al.*, 2021). In this relation it can be highlighted that a recent study found *Cyanobium* to be very sensitive to salt. With increasing concentrations of iron- and magnesium sulfate, *Cyanobium* was not able to survive (Ermis *et al.*, 2020). In this relation it is of particular importance that in the here presented study by Heitkamp *et al.* *Cyanobium* was only increased in the sludge fraction, but not on the biochar that was used as supplement, and which tends to adsorb salt and other inhibiting substances. Therefore, the study by Heitkamp *et al.* indicates that a combination of light and adsorptive materials could support the enrichment of Cyanobacteria such as *Cyanobium* in anaerobic digesters.

Besides H_2S, ammonia and ammonium are common inhibitors within anaerobic digestion. In general, ammonia and ammonium can only be degraded, when oxygen is present. Wastewater treatment plants therefore activate sludge with oxygen, which not only improves the efficiency in removing substances that are difficult to break down, but also reduces the number of potential pathogens (Aghalari *et al.*, 2020). An exception are so called ANAMMOX bacteria. Anammox bacteria can use nitrite or nitrate instead of oxygen for the oxidation of ammonium (Kuenen, 2008). Apart from ammonia oxidizing bacteria also ammonia oxidizing archaea are known (He et al., 2018). Therefore, the view that ammonia / ammonium oxidation takes place exclusively anaerobically has meanwhile been rejected. With the discovery of new ways of breaking down ammonium, one might wonder whether light could also play a role in its breakdown. Although only analyzed in freshwater, a recent study by French *et al.* (2012) describes that white and blue light seems to rather inhibit both, ammonium oxidizing bacteria and archaea. However, it must be considered that the microbial density in anaerobic digesters is much higher than in freshwater. Therefore, the involved microbial community might be very different. Also, DIET might play a role. Even if it has not yet been investigated and is very hypothetical, it would be conceivable that ammonia / ammonium oxidizing microorganisms are protected by pigmented microorganisms and receive electrons from them via conductive structures. In support of such a hypothetic syntrophic community, it can be emphasized that in particular syntrophic reaction communities seem to play an important role in the breakdown of nitrogen-rich substrates (Yan *et al.*, 2020). Although it is involved in nitrogen fixation rather than ammonia/ammonium breakdown, the recent characterization of the so-called iron-only nitrogenase should be emphasized. This nitrogenase could play an important role in such hypothetical electroactive, phototrophic communities. It can use electrons that are released from thiosulfate or the citric acid cycle to reduce carbon dioxide to fix nitrogen and to produce at the same time methane (Zheng *et al.*, 2018). Zheng *et al.* has shown this for the iron-only nitrogenase of the bacterium *Rhodopseudomonas palustris*. This article by Zheng *et al.* was not only an innovation in that it showed the possibility of bacterial methane formation, but it also indicates the metabolic complexity in phototrophic reactions. Moreover, the activity of the iron-only nitrogenase is coupled to carbon dioxide reduction, which might help to increase the methane ratio in anaerobic digesters. In this relation it is an outstanding discovery that a recent study describes

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a direct interspecies electron transfer between *Rhodopseudomonas palustris* and *Geobacter metallireducens*, as this directly links phototrophic activity to electroactivity (Liu *et al.*, 2021). In this relation it needs to be highlighted that the red biofilm, which was investigated by Abendroth et al. (2020) was enriched in the genus *Methanosarcina.* The importance of *Methanosarcina* for electroactive syntrophic communities and its relation to DIET has been explained in detail in the previous chapter. Therefore, the high ratio of *Methanosarcina* in phototrophic biofilms might indicate that there is a metabolic linkage between light harvesting microorganisms and electroactive, methanogenic archaea as well, although this has not been demonstrated so far. An increased ratio of *Methanosarcina* under the influence of light was 2006 already highlighted my Tada *et al.* (2006). While the study by Abendroth et al. (2020) was focused on a metagenomic characterization of the underlying biofilm, analyzed Tada *et al.* in detail the process performance of anaerobic digesters under the influence of blue light, stating an approximately 2,5-times higher methane productivity after 10 days.

In summary, it can be said: Even if the above assumptions are still hypothetical and further research is necessary, there is increasing evidence that the combination of light and electroactive microbiomes represents a realistic scenario for the cultivation of anaerobic high-performance microbiomes.

6.4.5 Flexible interaction patterns – and the question of the programmability of AD-microbiomes

Based on a recent article by Schwan *et al.* (2020), a futuristic and hypothetical outlook for anaerobic microbiomes will be presented at this point. Schwan *et al's* work takes up the question, which is raised with the title of this habilitation thesis: the question of the programmability of anaerobic microbiomes. Understanding microbial interactions and their influence on the composition of taxonomic profiles is important for this question. In publications 1-3 and 5 of this work, different strategies are presented how to influence anaerobic microbiomes. Strategies on how to manipulate anaerobic microbiomes are addressed in multiple research articles in different research fields. Examples ware works about aerobic granular sludge, the production of bioplastics and biofuels, the digestive system of humans and animals and about anaerobic ammonium oxidation (anammox). In this context the catchphrase "designer microbiomes" can now also be found in the literature (Strous and Sharp, 2018). With the advancing microbiome research, scientists started to investigate more and more influencing factors for the formation and manipulation of anaerobic microbiomes. This increasingly involves new innovative molecular biological approaches, such as high-throughput sequencing for RNA, DNA or proteins. Following this strategy, DNA high-throughput sequencing was used in almost all articles of this thesis. In the context of the present thesis, various influencing factors were discussed, such as pH, ammonium / ammonia, light and other. However, results that address the manipulability of anaerobic microbiomes are often based on an empirical adjustment of chemical and physical parameters of the AD black box and based on these results, attempts are made to find explanations for the effects that occur upon changing environmental conditions. A recently published study by Lim *et al.* (2020) en-

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titled "*The microbiome driving anaerobic digestion and microbial analysis*" illustrates the increasing efforts to understand anaerobic process biocenosis. Multiple techniques are mentioned, which are now applied, such as "*Metataxonomics, Metagenomics, Metaproteomics, Metametabolomics and Culturomics*". Many articles now use these methods and the data set on given microorganisms is growing immeasurably. However, considerations on microbial interactions are often not implemented or only shortly addressed. An exception are syntrophic relations, which is also the case for the study by Lim *et al.* (2020). Methods that are used to resolve syntrophic relations are for example isotope analysis, analysis of gene-expression and transcriptomics, electrochemical analyses, fluorescent in situ hybridization (microscopy), analysis for the redox state of cells b quantifying NAD+/NADH and ATP. Those methods were recently combined in an experimental approach to show the synthrophic relation between *Rhodopseudmonas palustris* and *Geobacter metallireducens* (Liu *et al.*, 2021). Another example is an article from Gorbi *et al.* (2006), which is one of the first articles dealing with the discovery of conductive nanowires. A combination of scanning- and transmission electron microscopy, scanning tunneling microscopy and tunneling spectroscopy, fluorescent microscopy, mutagenesis, ferrihydrite-reduction assays and MFC assays was necessary to give evidence for the direct interspecies electron transfer. In their work, Gorbi *et al.* have shown the ability for DIET *Shewanella oneidensis, Synechocystis PCC6803* and *Pelotomaculum thermopropionicum.* It was a highlight that the latter two can do this without being dissimilatory metal-reducing bacteria, which also highlights the importance of microbial nanowires for microbial interactions. Both works, that of Liu *et al.* (2021) and that of Golbi *et al.* (2006), worked with pure cultures and nevertheless a considerable methodological effort was necessary to prove the interactions of the organisms involved. If one considers the methodological effort that is necessary to detect microbial interactions between selected pure cultures, it seems almost impossible to resolve the interaction of all microorganisms in an AD microbiome. To give a couple of numbers here: Kirkegard *et al.* (2017) compared 32 full-scale biogas plant and found that the core microbiome comprises 300 OTUs. If microorganisms outside of the core-microbiome are considered as well, then the number increased into thousands. If the formula (($\frac{n!}{(n-k)!k!}$) is applied to 300 OTUs, which are forming the core-microbiome (n) and, for the sake of simplicity, limits the maximum number of possible interaction partners that interact with each other to 10 (k), there are $1.39832023324176 \times 10^{18}$ possible interaction variants. To study microbial interactions with so many possibilities, new approaches and methods are required. Many scientists have recognized this problem already and are trying to solve this problem based on sequencing results in combination with multivariate statistics. A good example is a recent study by De Vrieze *et al.* (2018). Co-occurrence networks were predicted, and functional predictions were possible. Correlation analyzes have shown a very diverse correlation pattern for the dependence of individual microorganisms on individual process parameters with the help of a heat map. For the respective microorganisms, very different correlations were found on the RNA and DNA level. Similar results were observed for the alpha- and beta diversity of the DNA and RNA, although the differences between DNA and RNA were much higher for Bacteria than for Archaea. De Vrieze *et al.* also applied non-metric distance scaling (NMDS) based on UniFrac distances, which is not a measure for the abundance, but for the relative relatedness, which includes phylogenetic distances. NMDS resulted in the finding that DNA and RNA profiles clustered far away from each other in the case of bacteria. On the other hand, they were close to each

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other in the case of archaea. This statistical result was interpretated in such a way, that the functional community structure remains rather similar, while shifts occur especially in the community composition. This was explained by the hypothesis that multiple organisms can occupy same niches and that multiple organisms are engaged in many metabolic pathways at the same time. The Vrieze *et al.* explained further that the community composition depends strongly on multiple chemical process parameters. Using a so called “canonical correspondence analysis” in combination with a “PERMANOVA”, it was revealed that the archaeal community is primarily shaped by temperature, pH, TAN, free ammonia, conductivity, VS and TS, although Na^+, K^+, propionate and total VFA had a strong impact as well. The bacterial community behaved slightly differently, with temperature, pH, TAN, free ammonia, conductivity, Na^+, K^+, VS and propionate being the main factors. Although less strong, there were other important factors, which were TS, Mg^{2+}, acetate, butyrate and total VFA. Although hard to comprehend for non-statisticians, works such the one from De Vrieze *et al.* are crucial to get a deeper understanding of anaerobic microbiomes. There are other studies, which are also applying multivariate statistics on AD processes and especially co-occurrence studies are used to predict microbial clusters of microorganisms, which might be physically or metabolically integrated with each other. A few exemplary studies that can be mentioned here come from Rui et al., (2015), Ziels *et al.* (2018) or Orellana *et al.* (2019). Some of these studies are not just performing co-occurrence studies based on 16S-rRNA gene amplicon sequences, but they include metagenomes, which was for example the case in the study by Orellana *et al.* (2019). Involving metagenomes allows the interpretation of the underlying metabolic pathways. Since the same genes in different organisms often show slight deviations in their DNA sequence, it is even possible to assign genes to specific organisms. With bioinformatic databases, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG), it can finally be checked how complete metabolic pathways are mapped by the respective organisms. This knowledge can in turn be used to check which organisms could complement each other at the metabolic level. Applying multivariate statistics on metagenomic data sets, Orellana *et al.* has shown for example, that there are two main clusters in household biogas digesters. At the functional level, cluster I was dominated by acetotrophic processes and by primary, *Clostridium*-associated fermentation processes. The second cluster was dominated by acetogenic and hydrogenotrophic processes, as well as primary fermentation processes by Spirochaetes, Bacteroidales, and Clostridia.

In addition to experimentally quite complex studies in pure culture and multivariate comparative analyzes based on metagenomes and 16S rRNA sequences, it is also a sensible option to include computer models of population dynamics in evaluations of sequence data. However, hardly any research results have been published in relation to biogas production. Two articles were found that presented online tools that allow the implementation of 16S-rRNA gene amplicon sequences in the so-called Lotka-Volterra model (Shaw *et al.*, 2016; Kuntal *et al.*, 2019. The Lotka-Volterra model is a model that predicts predator-prey interactions and is generally known in respect to the “rabbit-wolf graph”. Taking into account the fact that the tools for applying the Lotka-Volterra model to sequence data have been available since 2016, it is surprising that this tool has so far hardly been used in the biogas sector. To close this gap, the article 4 of the present thesis (Schwan *et al.*, 2020) has been published, which can now be regarded as one of the first articles that apply the Lotka-Volterra model in relation to biogas production. The special feature of this model is that the formula contains

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a microbial interaction coefficient, which indicates whether microorganisms behave more competitively or more symbiotically.

At this point it should be emphasized again that works like that of De Vrieze *et al.*, or Orellana *et al.* focus on statistical interpretations of empirical process fluctuations. One can ask now whether all these interaction studies based on pure cultures or high-throughput characterizations like the one from De Vrieze *et al.* allow us now to manipulate anaerobic microbiomes in a more targeted manner? At least they give us a glimpse. In previous chapters of the present thesis, various influencing factors were discussed, which were analyzed with high-throughput data or pure-culture approaches as well. The combined interpretation of all these studies might enable a new function of anaerobic microbiomes: The combination of conductive particles, light, low viscosity, low pH, high concentrations of COD, VFAs, salt and ammonia could give rise to formation of phototrophic, electroactive high-performance microbiomes, although this possibility has not yet been proven.

Another approach, which is also somewhat more targeted than the mere variation of process parameters, is the addition of microorganisms enriched separately in pure culture (bio-augmentation). In this relation, an interesting approach was published by Kovács *et al.* (2013). As hydrogenotrophic archaea are important for methane formation, the hydrogen forming bacteria *Caldicellulosiruptor saccharolyticus* and *Enterobacter cloacae* were added to lab-scale digester experiments. Kovács *et al.* observed a higher biogas productivity, which surprises as higher levels of hydrogen might also inhibit syntrophic relations. Although in case of the hydrogen forming bacterium *Enterobacter cloacae* a long-lasting effect with a stable cell number was observed (Ács et al., 2015), bioaugmentation is a process with a questionable / uncertain outcome. A good example of this is a work by Strang *et al.* (2017), which enriched a cellulose consortium from a biogas process that increased methane formation by 22-24% in augmented biogas reactors. After the 4 most common species (*Thermoanaerobacterium thermosaccharolyticum, Caldanaerobacter subterraneus, Thermoanaerobacter pseudethanolicus* and *Clostridium cellulolyticum*) were isolated and enriched, the positive effects in augmented reactors were reduced by half compared to the effects by the original consortium. This shows that the metabolic complexity is still beyond our understanding. The work from Strang *et al.* also shows that even microorganisms that occur in very small quantities can make an important contribution.

Another important influencing factor is the competitiveness of the microorganisms involved. As already discussed in the introduction to this thesis, it is a probable scenario that added microorganisms are repressed by the native microorganisms of the underlying microbiome. In order to overcome this hurdle, it is of particular importance that selection pressures are used which, in particular, favor the added type of microorganisms. The following examples can be given here: Strang *et al.* (2017) added lignocellulolytic bacteria using lignocellulolytic biomass as selection pressure, as such biomass degrades only very slowly. In addition to lignocellulolytic biomass, fats are also poorly degradable. This can therefore also be seen as a selection pressure, which Cirne *et al.* (2006) used for bioaugmentation with fat-hydrolyzing bacteria. Another useful selection pressure is the slow rate of growth for syntrophic bacteria. Syntrophic bacteria are usually growing very slowly but are still of central importance for methanogenesis or the breakdown of fatty acids. Their supplementation can therefore reduce the long start-up phase of reactors. Especially at high ammonium concentrations, syntrophic reaction communities seem to be important. Westerholm *el al.* (2012) inoculated

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successfully syntrophic acetate-oxidizing bacteria into a process with high ammonia concentration. This has two advantages. The enrichment of slowly growing syntrophs is accelerated and at the same time fermentation is facilitated with high ammonium contents.

Based on the examples listed and the selection pressures on which they are based, it becomes clear that the majority of bioaugmentation attempts are aimed at increasing the degradation efficiency and / or overcoming process disturbances. Due to unfavorable process conditions or poor degradability, the required selection pressure is usually already given. Bioaugmentation can shorten the time it takes for the microbiome to adapt to the prevailing conditions, and it can also prevent the reactor from crashing prematurely.

The question arises as to whether targeted functional changes are possible without poor substrate degradability or without having process disturbances. In terms of direct electron transfer between species, this is certainly the case. A recent article from Salvador *et al.* (2017) indicates further that carbon-nanotubes improved digesting conditions without showing any sign of direct interspecies electron transfer. This highlights that apart from DIET there might be further and yet unknown effects on the molecular level, which might be used to refurbish future AD-microbiomes. At this point it should be emphasized again that anaerobic microbiomes represent a treasure trove that we have barely opened so far. Building on this fact, Kleeberezem *et al.* titled a 2015 published review article with the question: "Anaerobic digestion without biogas? ". Kleeberezem *et al.* mention multiple metabolites of high value: xylitol, glucaric acid, 1,2-propanediol, 1,3-proanediol, 3-hydroxypropionate, L-lactate, 2,3-butanediol, L-alanine, ethanol, butanol, acetone, butyrate, adipic acid, acetate, PHB, citrate, itaconate, L-glutamate, putrescine, succinate, fumarate, malate, caprolactam, cadaverine, L-isoleucine, L-methionine, L-threonine, L-lysine, L-asparate. It would be a significant innovation if we could manipulate microbiomes to produce selected metabolites of the above as needed. To achieve this, we need completely new approaches to program and neofunctionalize microbiomes. And for this neofunctionalization a deeper understanding of anaerobic microbiomes is needed. In this context, the results of the Lotka-Volterra modeling from publication 4 of this thesis are interesting (Schwan *et al.*, 2020). Starting from the same microbiome, several parallel experiments were carried out. These experiments were subjected to different chemical stressors. For some of the most abundant microorganisms, the interaction coefficient was determined from the Lotka-Volterra equation. Interestingly, it turned out that the interaction coefficient can indicate competitive or symbiotic relationships, or no relationship at all, between two given species, depending on the particular stressor. If the question of the neofunctionalization of anaerobic microbiomes is raised, this finding is important because it shows that a certain combination of microorganisms to be added in combination with varied selection pressures may only function under very defined reactor conditions. Apart from the fact that Schwan's work further complicates the high complexity of anaerobic microbiomes, a new approach to microbiome manipulation can also be devised based on the Lotka-Volterra model. If a certain target organism is introduced into a microbiome, Lotka-Volterra modeling based on 16S rRNA data could be used to search for microorganisms that have a strongly positive interaction coefficient with the target organism. The corresponding microorganism could now also be enriched. This "accompanying" organism could now be co-inoculated with the target organism. This method is hypothetical at first and should be explored further in the future. Should it prove to be true, this could considerably increase the chances of success in domesticating microorganisms in an alien environment.

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6.5 Pre-treatment of substrates

As already highlighted in the previous sections, a two-stage anaerobic biogas process with an acidic preliminary stage is the key to the enrichment of metabolites, which could be of central importance for the growing bioeconomy. A recent article by Ramm et al. (2020) is focused on N-removal technologies from high-strength liquor received from dark fermentation (two-stage methanation). The results presented in the work from Ramm *et al.* (2020) show that both MAP precipitation and N-stripping are suitable methods for the treatment of acidic high strength liquors resulting from substrate pretreatment from two-stage digesters. At this point the legitimate question could be asked whether N-stripping or MAP precipitation can also be used economically. In this relation it can be pointed out that similar N-removal technologies as described by Ramm et al. are already being used in industry, although they are only applied in separate fermentation stages. In this relation, a recent online statement by the European Biogas Association (EBA) says: "*The Green Energy farm holding of Chiari (BS – Italy) is an example of a success story. In fact, the company has a 1MW biogas plant mainly fed with chicken droppings and connected to a BTS Biogas stripping system which has made it possible to reduce nitrogen levels by up to 60%.*". The mentioned company is just one of multiple companies that can be found in this relation. In this context, however, it should also be noted that the maintenance costs for N-elimination technologies in acidification stages could possibly turn out differently than they are for methane stages. But still, the metabolites that might be retrieved from dark fermentation stages are much more valuable than just methane. A comparison of the monetary value of different metabolites was done by Kleerebezem *et al.* (2015). To improve comparability, Kleerebezem *et al.* related the gain to the electrons that are contained in the respective metabolites. In June 2013, methane had a value of 0.8 EUR $kmol_{-e}^{-1}$. In contrast, hydrogen or ethanol would both yield 0.8 EUR $kmol_{-e}^{-1}$, and poly-hydroxybutyrate (PHB) would even yield 12.4 EUR $kmol_{-e}^{-1}$, which is already 15.5-times as much revenue as from methane. Although this was not demonstrated yet, the high revenue from other metabolites than methane makes it appear likely that possible extra costs for the building and maintenance of a respective dark fermentation stage would be covered by the higher revenue obtained for the respective metabolites.

In this relation someone could ask why the N-elimination in two-stage systems should be carried out in the acidification stage and not in the methane stage as it is already done. This question cannot be answered with absolute certainty, but at least some clues from the current literature are given. First of all, the proposed technology from Ramm *et al.* was able to increase the acid formation by up to 19%. If one considers the increased revenues for the metabolites of dark fermentation compared to methane, 19% additional yield can already make a big monetary difference. In this relation, it is important to note that the proposed technology did not only improve the conditions for bacteria due to lowered nitrogen contents, but it is possible to selectively inhibit methanogenic archaea. With inhibited methanogens the long-term stability of the high-strength liquor produced might be improved as well. Another advantage, which has not yet been investigated in this context, is increased hydrogen formation. Many publications show that harsh conditions, in particular temperature

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shocks, can increase hydrogen formation, as for example shown in the works from Magrini *et al.* (2020) and Alibardi *et al.* (2012).

It was also shown recently that magnesium can increase the efficiency for hydrogen formation, as it is an important co-factor for many enzymes. Cardoso *et al.* (2014) found the hydrogen formation to be optimal at magnesium concentrations between 1.2 and 1.6 g L^{-1}. As magnesium is one of the substances added for MAP precipitation (Ramm *et al.* applied 89 g L^{-1} of magnesium chloride hexahydrate), this method could also increase the magnesium content in a separated acidification stage. Unfortunately, the magnesium contents as not measured after the application of the MAP precipitation in the high-strength liquor produced. However, it was noted that that concentration of all trace elements that were measured decreased, and that in parallel the conductivity increased. This indicated that the MAP precipitation increases the concentration of magnesium during the fermentation process.

It could also be advantageous to use larger amounts of sodium hydroxide (50 g L^{-1}) during the N-stripping to shift the pH value to a more basic level. A recent study by Salmón *et al.* (2018) has shown that alkaline treatments are well suited for CO_2 capture with carbonate produced simultaneously. Therefore, the N-removal technology suggested by Ramm *et al.* might also be used for the purification of produced hydrogen, although this has not been experimentally tested so far.

Following the hypothesis that the proposed N-removal technology in the present study could be used to increase the hydrogen formation, this would in turn affect the acid formation as well. It is known that the hydrogen partial pressure in the gas phase affects the ratio of different VFAs in the process. Higher partial pressures of hydrogen inhibit the hydrogenase enzyme, which in turn changes the metabolism, resulting in higher levels of butyric acid (Dwidar *et al.*, 2012). Although butyric acid is a wanted and valuable product for the industry, as described by Dwidar *et al.*, in higher concentrations it affects methanogenic fermentation negatively (Kroiss and Svarland, 2005). The literature describes several technologies that allow efficient separation of VFAs from fermentation processes, such as ion exchange processes, solvent extraction, electrodialysis (Murali *et al.*, 2017) or adsorption (Reyhanitash *et al.*, 2017) processes. Yousuf *et al.* (2016) tried to improve the separation process or VFAs, reaching up to 74% of removal efficiency. But even with such high efficiencies, a substantial amount of butyric acid might enter the subsequent methanation stage that is used to digest the residues from the fermentation. Therefore, to improve the methanogenic digestibility of fermentation residues that are enriched in propionic- or butyric acid, further improvements are necessary. One possible solution could be the enrichment of *Methanosarcina* in combination with an increased rate of direct interspecies electron transfer (DIET). This idea is extensively discussed in the following chapters.

References

- Abendroth C, Vilanova C, Günther T, Luschnig O, Porcar M. 2015. Eubacteria and archaea communities in seven mesophile anaerobic digester plants in Germany. Biotechnolo Biofuels. 8:87.
- Abendroth C, Wünsche E, Luschnig O, Bürger C, Günther T. 2015. Producing high-strength liquor from mesophilic batch acidification of chicken manure. Waste Manag Res. 33, 291 – 294.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Abendroth C, Simeonov C, Peret J, Antnez O, Gavidia R, Luschnig O, Porcar M. 2017a. From grass to gas: microbiome dynamics of grass biomass acidification under mesophilic and thermophilic temperatures. Biotechnol Biofuels 10:171.
- Abendroth C. 2018. Paving the crossroad of biorefinery. PhD thesis, University of Valencia, Spain.
- Abendroth C, Latorre-Pérez A, Porcar M, Simeonov C, Luschnig O, Vilanova C, Pascual J. 2020. Shedding light on biogas: Phototrophic biofilms in anaerobic digesters hold potential for improved biogas production. Syst Appl Microbiol. 43, 126024.
- Ács N, Bagi Z, Rákhely G, Minárovics J, Nagry K, Kovács KL. 2015. Bioaugmentation of biogas production by a hydrogen-producing bacterium. Bioresour Technol. 186, 286-293.
- Aghalari Z, Dahms HU, Sillanpää M, Sosa-Hernandez JE, Parra-Saldívar R. 2020. Effectiveness of wastewater treatment systems in removing microbial agents: a systematic review. Global Health. 16:13.
- Alibardi L, Favaro L, Lavagnolo MC, Basaglia M, Casella S. 2012. Effects of heat treatment on microbial communities of granular sludge for biological hydrogen production. Water Sci Technol. 66, 1483–1490.
- Al Seadi T, Rutz D, Prassl H, Köttner M, Finsterwalder T, Volk S, Janssen R. 2008. "Biogas Handbook, ed. Al Seadi, T. (University of Southern Denmark), ISBN 978-87-992962-0-0.
- Artegoitia VM, Foote AP, Lewis RM, Freetly HC. 2017. Rumen Fluid Metabolomics Analysis Associated with Feed Efficiency on Crossbred Steers. Sci Rep. 7:864.
- Barber WP, Stuckey DC. 1999. The use of the anaerobic baffled reactor (ABR) for wastewater treatment: a review. Water Research. 33, 1559-1578.
- Bargiela R, Herbst FA, Martínez-Martínez M, Seifert J, Rojo D, Cappello S, Genovese M, Crisafi F, Denaro R, Chernikova TN, Barbas C, Von Bergen M, Yakimov MM, Ferrer M, Golyshin PN. 2015. Metaproteomics and metabolomics analyses of chronically petroleum-polluted sites reveal the importance of general anaerobic processes uncoupled with degradation. Proteomics. 15, 3508–3520.
- Becker D, Popp D, Harms H, Centler F. 2020. A Modular Metagenomics Pipeline Allowing for the Inclusion of Prior Knowledge Using the Example of Anaerobic Digestion. Microorganisms. 8:669.
- Beckmann S, Welte C, Li X, Oo YM, Kroeninger L, Heo Y, Zhang M, Ribeiro D, Lee M, Bhadbhade M, Marjo CE, Seidel J, Deppenmeier U, Manefield M. 2016. Novel phenazine crystals enable direct electron transfer to methanogens in anaerobic digestion by redox potential modulation. Energy Environ Sci. 9:644.
- Berg G, Rybakova D, Fischer D, Cernava T, Champomier Vergè MC et al. 2020. Microbiome definition re-visited: old concepts and new challenges. Microbiome 8:103.
- Bertucci M, Calusinska M, Goux X, Rouland-Lefèvre C, Untereiner B, Ferrer P, Gerin PA, Delfosse P. 2019. Carbohydrate Hydrolytic Potential and Redundancy of an Anaerobic Digestion Microbiome Exposed to Acidosis, as Uncovered by MetagenomicsAppl Environ Microbiol. 85:e00895-19.
- Botheju D, Bakke R. 2011. Oxygen Effects in Anaerobic Digestion – A Review. J Waste Manag. 4, 1-19.
- Buan N, Kulkarni G, Metcalf W. 2011. Genetic methods for *Methanosarcina* species. In: Methods in Methane Metabolism, Part A. Rosenzweig, A., Ragsdale, S., (eds.); Methods in Enzymology 494, ISBN: 9780123851123.
- Cardoso V, Romao BB, Silva FTM, Santos JG, Batista FRX, Ferreira JS. 2014 Hydrogen production by dark fermentation, Chem Eng Transact. 38, 481-486.
- Chen WS, Strik DPBTB, Buisman CJN, Kroeze C. 2017. Production of Caproic Acid from Mixed Organic Waste: An Environmental Life Cycle Perspective. Environ Sci Technol. 51, 7159–7168.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Chen WY, Kraková L, Wu JH, Pangallo D, Jeszeová L, Liu B, Yasui H. 2017. Community and Proteomic Analysis of Anaerobic Consortia Converting Tetramethylammonium to Methane. Archaea, 2170535.
- Cirne DG, Björnsson L, Alves M, Mattiasson B. 2006. Effects of bioaugmentation by an anaerobic lipolytic bacterium on anaerobic digestion of lipid-rich waste. Chem Tech Biotechnol. 81, 1745-1752.
- Cohen Y, Jørgensen BB, Revsbech NP, Poplawski R. 1986. Adaptation to Hydrogen Sulfide of Oxygenic and Anoxygenic Photosynthesis among Cyanobacteria. Appl Environ Microbiol. 51, 398-407.
- Costa A, Tangorra FM, Zaninelli M, Oberti R, Cavalchini AG, Savoini G, Lazzari M. 2016. Evaluating an e-nose ability to detect biogas plant efficiency: a case study, Italian Journal of Animal Science, 15:1,116-123.
- Dai X, Yan H, Li N, He J, Ding Y, Dai L, Dong B. 2016. Metabolic adaptation of microbial communities to ammonium stress in a high solid anaerobic digester with dewatered sludge. Sci Rep. 6:28193.
- De Groof V, Coma M, Arnot T, Leak DJ, Lanham AB. 2019. Medium Chain Carboxylic Acids from Complex Organic Feedstocks by Mixed Culture Fermentation. Molecules. 24:398.
- De Vrieze J, Hennebel T, Boon N, Verstraete W. 2012. *Methanosarcina*: The rediscovered methanogen for heavy duty biomethanation. Bioresour Technol. 112, 1 – 9.
- De Vrieze J. 2014. *Methanosaeta* vs. *Methanosarcina* in anaerobic digestion: the quest for enhanced biogas production. PhD thesis, Ghent University, Belgium.
- De Vrieze J, Pinto AJ, Sloan WT, Ijaz UZ. 2018. The active microbial community more accurately reflects the anaerobic digestion process: 16S rRNA (gene) sequencing as a predictive tool. Microbiome. 6:63.
- Dick GJ, Grim SL, Klatt JM. 2018. Controls on O_2 Production in Cyanobacterial Mats and Implications for Earth's Oxygenation. Annu. Rev. Earth Planet. Sci. 46:123–47.
- Dwidar M, Park JY, Mitchell RJ, Sang BI. 2012.The Future of Butyric Acid in Industry. Sci World J. 471417.
- EBA (European Biogas Association): https://www.europeanbiogas.eu/eba-statistical-report-2020/ (accessed 23.06.2021).
- EBA (European Biogas Association): https://www.europeanbiogas.eu/bts-latest-biogas-stripping-system-is-able-to-remove-up-to-70-of-the-nitrogen-present-in-the-digestate-produced-by-biogas-plants/ (accessed 26.06.2021).
- Eduok S, Ferguson R, Jefferson B, Villa R, Coulon F. 2017. Aged-engineered nanoparticles effect on sludge anaerobic digestion performance and associated microbial communities. Sci Total Environ. 609, 232 – 241.
- Ermis H, Guven-Gulhan U, Cakir T, Altinbas M. 2020. Effect of iron and magnesium addition on population dynamics and high value product of microalgae grown in anaerobic liquid digestate. Sci Rep. 10:3510.
- Fang HHP, Yeong CLY, Book KM, Chiu CMM. 1993. Removal of COD and nitrogen in wastewater using sequencing batch reactor with fibrous packing. Water Sci Tech. 28, 125 – 131.
- French E, Kozlowski JA, Mukherjee M, Bullerjahn G, Bollmann A. 2012. Ecophysiological Characterization of Ammonia-Oxidizing Archaea and Bacteria from Freshwater. Appl Environ Microbiol. 78, 5773-5780.
- Frigaard NU. 2016. Biotechnology of anoxygenic phototrophic bacteria. Adv Biochem Eng Biotechnol. 156:139-154.
- Frigaard NU. 2016. Biotechnology of anoxygenic phototrophic bacteria. Adv Biochem Eng Biotechnol. 156:139-154.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Garcia ML, Angenent LT. 2009. Interaction between temperature and ammonia in meso-philicdigesters for animal waste treatment. Water Research. 43, 2373-2382.
- Garrity GM, Labeda DP, Oren A. Judicial Commission of the International Committee on Systematics of Prokaryotes. XIIth International (IUMS) Congress of Bacteriology and Applied Microbiology. Minutes of the meetings, 3, 4 and 6 August 2008, Istanbul, Turkey. Int J Syst Evol Microbiol., 2011, 61, 2775-2780.
- Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim SK, Culley DE, Reed SB, Romine MF, Saffarini DA, Hill EA, Shi L, Elias DA, Kennedy DW, Pinchuk G, Watanabe K, Ishii S, Logan B, Nealson KH, Fredrickson JK. 2006. Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. Proceedings of the National Academy of Sciences of the USA 103, 11358–11363.
- Gruninger RJ, Nguyen TTM, Reid ID, Yanke JL, Wang P, Abbott DW, Tsang A, McAllister T. 2018. Application of Transcriptomics to Compare the Carbohydrate Active Enzymes That Are Expressed by Diverse Genera of Anaerobic Fungi to Degrade Plant Cell Wall Carbohydrates. Front Microbiol. 9:1581.
- Gunnarsson IB, Alvarado-Morales M, Angelidaki I. 2014. Utilization of CO2 fixating bacterium Actinobacillus succinogenes 130Z for simultaneous biogas upgrading and biosuccinic acid production. Environ Sci Technol. 48:12464-8.
- Hardegen J. 2017. Influence of the quantity of methanogenic archaea on the process performance of anaerobic digester plants. Bachelor thesis, University of Heidelberg.
- Hanada S. 2016. Anoxygenic Photosynthesis —A Photochemical Reaction That Does Not Contribute to Oxygen Reproduction—. Microbes Environ. 31, 1-3.
- Heitkamp K, Latorre-Pérez A, Nefigmann S, Gimeno-Valero H, Vilanova C, Jahmad E, Abendroth C, 2021. Monitoring of seven industrial anaerobic digesters supplied with biochar. Biotechnol Biofuels. 14:185.
- He H, Zhen Y, Mi T, Fu L, Yu Z. 2018. Ammonia-Oxidizing Archaea and Bacteria Differentially Contribute to Ammonia Oxidation in Sediments from Adjacent Waters of Rushan Bay, China. Front Microbiol. 9:116.
- Holmes DE, Orelana R, Giloteaux L, Wang LY, Shrestha P, Williams K, Lovley DR, Rotaru AE. 2018. Potential for *Methanosarcina* to Contribute to Uranium Reduction during Acetate-Promoted Groundwater Bioremediation. Microb Ecol. 76, 660 – 667.
- Jetten MSM, Stams AJM, Zehnder AJB. 1991. Methanogenesis from acetate: a comparison of the acetate metabolism in Methanothri¢ soehngenii and Methanosarcina spp. FEMS Microbiol Rev. 88, 181-198.
- Kadier A, Simayi S, Abdeshahian P, Azman NF, Chandrasekhar K, Kalila MS. 2016. A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production. Alex Eng J. 55, 427-443.
- Kasali GB, Senior E, Watson-Craik IA. 1989. Sodium bicarbonate effects on the anaerobic digestion of refuse. J Chem Technol Biotechnol. doi: 10.1002/jctb.280450405.
- Kashket ER. 1985. The proton motive force in bacteria: a critical assessment of methods. Ann. Rev. Micro. 39, 219-242.
- Kato S, Hashimoto K, Watanabe K. 2012. Methanogenesis facilitated by electric syntrophy via (semi)conductive iron-oxide minerals. Environ Microbiol. 14, 1646 – 1654.
- KEGG, map0068 - https://www.genome.jp/dbget-bin/www_bget?pathway:map00680 (accessed 23.06.2021)
- Kern T, Fischer MA, Deppenmeier U, Schmitz RA, Rother M. 2016. *Methanosarcina flavescenssp*. nov., a methanogenic archaeon isolated from a full-scaleanaerobic digester. Int J Syst Evol Microbiol. 66, 1533–1538.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Kirkegaard RH, McIlroy SJ, Kristensen JM, Nierychlo M, Karst SM, Dueholm MS, Albertson M, Nielsen PH. 2017. The impact of immigration on microbial community composition in full-scale anaerobic digesters. Sci. Rep. 7:9343.
- Kleerebezem R, Joosse B, Rozendal R, Van Loosdrecht MCM. 2015. Anaerobic digestion without biogas? Rev Environ Sci Biotechnol. 14, 787–801.
- Kovács KL, Ács N, Kovács E, Wirth R, Rákhley G, Strang O, Herbel Z, Bagi Z. 2013. Improvement of biogas production by bioaugmentation. Biomed Res Int. 2013:482653.
- Kroiss H, Svarland K. 2005. Einflussfaktoren auf die anaeroben biologischen Abbauvorgange, In: Bischofsberger W, Dichtl N, Rosenwinkel KH, Seyfried CF, Böhnke B. (eds.). Anaerobtechnik. (Springer) ISBN 3-540-06850-3.
- Kroiss H, Svarland K. 2005. Einflussfaktoren auf die anaeroben biologischen Abbauvorgange, In: Bischofsberger W, Dichtl N, Rosenwinkel KH, Seyfried CF, Böhnke B. (eds.). Anaerobtechnik. (Springer) ISBN 3-540-06850-3.
- Kuenen JG. 2008. Anammox bacteria: from discovery to application. Nat Rev Microbiol. 6, 320–326.
- Kuntal B, Gadgil C, Mandel SS. 2019. Web-gLV: A Web Based Platform for Lotka-Volterra Based Modeling and Simulation of Microbial Populations. Front Microbiol. 10:288.
- Laczi K, Kis AE, Szilágyi Á, Bounedjoum N, Bodor A, Vincze GE, Kovács T, Rákhely G, Perei K. 2020. New Frontiers of Anaerobic Hydrocarbon Biodegradation in the Multi-Omics Era. Front Microbiol. 2020; 11: 590049.
- Lackner N, Hintersonnleitner A, Wagner AO, Illmer P. 2018. Hydrogenotrophic Methanogenesis and Autotrophic Growth of *Methanosarcina thermophila*. Archaea. 2018:4712608.
- Lili M, Biró G, Sulyok E, Petis M, Borbély J, Tamás J. 2011. Conference: International Symposium "Risk Factors for Environment and Food Safety" & "Natural Resources and Sustainable Development" & "50 Years of Agriculture Research in Oradea", Volume: 802-807
- Lin Y, Lü F, Shao L, He P. 2013. Influence of bicarbonate buffer on the methanogenetic pathway during thermophilic anaerobic digestion. Bioresour Technol. 137, 245-253.
- Lim JW, Park T, Tong YW, Yu Z. 2020. The microbiome driving anaerobic digestion and microbial analysis. Adv Bioenerg. 5, 1-61.
- Liu H, Grot S, Logan BE. 2005. Electrochemically assisted microbial production of hydrogen from acetate, Environ. Sci. Technol. 39, 4317–4320.
- Liu F, Rotaru AE, Shrestha PM, Malvankar NS, Nevin KP, Lovley DR. 2012. Promoting direct interspecies electron transfer with activated carbon. Energy Environ. Sci. 5, 8982 – 8989.
- Martins G, Salvador AF, Pereira L, Alves MM. 2018. Methane Production and Conductive Materials: A Critical Review. Environ Sci Technol. 52, 10241-10253.
- Liu X, Huang L, Rensing C, Ye J, Nealson KH, Zhou S. 2021. Syntrophic interspecies electron transfer drives carbon fixation and growth by *Rhodopseudomonas palustris* under dark, anoxic conditions. Sci Adv. 7 : eabh1852.
- Maeder DL, Anderson I, Brettin TS, Bruce DC, Gilna P, Han CS, Lapidus A, Metcalf WW, Saunders E, Tapia R, Sowers KR. 2006. The *Methanosarcina barkeri* Genome: Comparative Analysis with *Methanosarcina acetivorans* and *Methanosarcina mazei* Reveals Extensive Rearrangement within *Methanosarcinal* Genomes. J Bacteriol. 188, 7922–7931.
- Magrini FE, de Almeida GM, da Maia Soares D, Fuentes L, Ecthebehere C, Beal LL, Da Silveira MM, Paesi S. 2020. Effect of different heat treatments of inoculum on the production of hydrogen and volatile fatty acids by dark fermentation of sugarcane vinasse. Biomass Conv. Bioref. https://doi.org/10.1007/s13399-020-00687-0.
- Martins G, Salvador AF, Pereira L, Alves MM. 2018. Methane Production and Conductive Materials: A Critical Review. Environ Sci Technol. 52. 10241 – 10253.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Maus I, Koeck DE, Cibis KG, Hahnke S, Kim YS, Langer T, Kreubel J, Erhard M, Bremges A, Off S, Stolze Y, Jaenicke S, Goesmann A, Sczyrba A, Scherer P, König H, Schwarz WH, Zverlov VV, Liebl W, Pühler A, Schlüter A, Klocke M. 2016. Unraveling the microbiome of a thermophilic biogas plant by metagenome and metatranscriptome analysis complemented by characterization of bacterial and archaeal isolates. Biotechnol Biofuels. 9:171.
- McKeown RM, Scully C, Enright AM, Chinalia FA, Lee C, Mahony T, Collins G, O'Flaherty V. 2009. Psychrophilic methanogenic community development during long-term cultivation of anaerobic granular biofilms. ISME. 3, 1231-1242.
- Montusiewicz A, Szaja A, Musielewicz I, Cydzik-Kwiatkowska A, Lebiocka M. 2020. Effect of bioaugmentation on digestate metal concentrations in anaerobic digestion of sewage sludge. PLoS One. 2020; 15:e0235508.
- Mulat DG, Huerta SG, Kalyani D, Horn SJ. 2018. Enhancing methane production from lignocellulosic biomass by combined steam-explosion pretreatment and bioaugmentation with cellulolytic bacterium *Caldicellulosiruptor bescii*. Biotechnol Biofuels. 11:19.
- Müller R, Mahro B. 2001. Bioaugmentation: Advantages and Problems Using Micro Organisms with Special Abilities in Soil Decontamination. In: Stegmann R, Brunner G, Calmano W, Matz G. (eds.). Treatment of Contaminated Soil. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-04643-2_21.
- Murali N, Srinivas K, Ahring BK. 2017. Biochemical Production and Separation of Carboxylic Acids for Biorefinery Applications. Fermentation. 3:22.
- Mutschlechner M, Praeg N, Illmer P. 2020. Soil-Derived Inocula Enhance Methane Production and Counteract Common Process Failures During Anaerobic Digestion. Front Microbiol. 11:572759.
- NCBI - https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi (accessed 30.10.2018) (NCBI:txid2207).
- Neumann H. 2021. Ausschreibung: EEG 2021 trifft die Biogasbranche mit voller Wucht. https://www.topagrar.com/energie/news/ausschreibung-eeg-2021-trifft-die-biogasbranche-mit-voller-wucht-12558112.html (accessed 23.06.2021).
- Nsair A, Onen Cinar S, Alassali A, Abu Qdais H, Kuchta K. 2020. Operational Parameters of Biogas Plants: A Review and Evaluation Study. Energies, 13:3761.
- Orellana E, Davies-Sala C, Guerrero LD, Vardé I, Altina M, Lorenzo MC, Figuerola EL, Pontiggia RM, Erijman L. 2019. Microbiome network analysis of co-occurrence patterns in anaerobic co-digestion of sewage sludge and food waste. Water Sci Technol. 79, 1956-1965.
- Oshurkova V, Troshina O, Trubitsyn V, Ryzhmanova Y, Bochkareva O, Shcherbakova, V. 2020. Characterization of Methanosarcina mazei JL01 Isolated from Holocene Arctic Permafrost and Study of the Archaeon Cooperation with Bacterium *Sphaerochaeta associata GLS2T*. Proceedings. 66, 4.
- Park J, Lee B, Shin W, Jo S, Jun H. 2018. Psychrophilic methanogenesis of food waste in a bio-electrochemical anaerobic digester with rotating impeller electrode. J Clean Prod. 188, 556-567.
- Pei Y, Yu Z, Ji J, Khan A, Li X. 2018. Microbial Community Structure and Function Indicate the Severity of Chromium Contamination of the Yellow River. Front Microbiol. 9:38.
- Poirier S, Déjean S, Chapleur O. 2018. Support media can steer methanogenesis in the presence of phenol through biotic and abiotic effects. Water Res. 140, 24 – 33.
- Ramm P, Abendroth C, Latorre-Pérez A, Herrmann C, Sebök S, Geißler A, Vilanova C, Porcar M, Dornack C, Bürger C, Schwarz H, Luschnig O. 2020. Ammonia removal during leach-bed acidification leads to optimized organic acid production from chicken manure. Renew Energ. 146, 1021-1030.
- Reguera G, McCarthy KD, Mehta T, Nicoll J, Tuominen MT, Lovley DR. 2005. Extracellular electron transfer via microbial nanowires. Nature. 435, 1098–1101.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Reguera G, Nevin KP, Nicoll JS, Covalla SF, Woodard T, Lovley DR. 2006. Biofilm and nanowire production leads to increased current in *Geobacter sulfurreducens* fuel cells. Applied and Environmental Microbiology. 72, 7345–7348.
- Reyhanitash E, Kersten SRA, Schuur B. 2017. Recovery of volatile fatty acids from fermented wastewater by adsorbtion. ACS Sustainable Chem Eng. 5, 9176-9184.
- Rhoades R A, Bell DR. 2012. Medical physiology: principles for clinical medicine (4th ed., international ed.). Philadelphia, Pa.: Lippincott Williams & Wilkins. ISBN 9781451110395.
- Robles G, Nair RB, Kleinsteuber S, Nikolausz M, Horváth IS. 2018. "Biogas Production: Microbiological Aspects" in Biogas: Fundamentals, Process and Operation; Biogas, Biofuel and Biorefinery Technologies 6, ed. Tabatabaei, M., Ghanavati, H. (Springer Nature), doi: 10.1007/978-3-319-77335-3_7.
- Rotaru AE, Shrestha PM, Liu F, Markovaite B, Chen S, Nevin KP, Lovley DR. 2014. Direct Interspecies Electron Transfer between *Geobacter metallireducens* and *Methanosarcina barkeri*. Appl Environ Microbiol. 80, 4599 – 4605.
- Rozendal RA, Buisman CJN. 2005. Process for Producing Hydrogen, Patent WO2005005981.
- Rui J, Li J, Zhang S, Yan X, Wang Y, Li X. 2015. The core populations and co-occurrence patterns of prokaryotic communities in household biogas digesters. Biotechnol Biofuels 8, 158.
- Salmón IR, Cambier N, Luis P. 2018. CO_2 Capture by Alkaline Solution for Carbonate Production: A Comparison between a Packed Column and a Membrane Contactor. Appl Sci. 8:996.
- Salvador AF, Martins G, Melle-Franco M, Serpa R, Stams AJM, Cavaleiro AJ, Pereira MA, Alves MM. 2017. Carbon nanotubes accelerate methane production in pure cultures of methanogens and in a syntrophic coculture. Environ Microbiol. 19, 2727–2739.
- Schwan B, Abendroth C, Latorre-Pérez A, Porcar M, Vilanova C, Dornack C. 2020. Chemically Stressed Bacterial Communities in Anaerobic Digesters Exhibit Resilience and Ecological Flexibility. Front Microbiol. 11:867.
- Shaw GTW, Pao YY, Wang D. 2016. MetaMIS: a metagenomic microbial interaction simulator based on microbial community profiles. BMC Bioinformatics. 17:488.
- Schink B, Stams AJM. 2006. Syntrophism among Prokaryotes. In: Dworkin M, Falkow S, Rosenberg E, et al. (eds) The Prokaryotes: Volume 2: Ecophysiology and Biochemistry. New York, NY: Springer New York, pp.309-335.
- Sieber JR, McInerney MJ, Gunsalus RP. 2012. Genomic insights into synthrophy: the paradigm for anaerobic metabolic cooperation. Annu Rev Microbiol. 66, 429 – 452.
- Selzer P, Marhöfer RJ, Koch R. 2018. Applied Bioinformatics – An Introduction, 2nd edition, Springer, Cham (Switzerland), ISBN 978-3-319-68301-0, doi: /10.1007/978-3-319-68301-0.
- Shimizu S, Upadhye R, Ishijima Y, Naganuma T. 2011*. Methanosarcina horonobensissp*. nov., amethanogenic archaeon isolated from a deepsubsurface Miocene formation. Int J Syst and Evol Microbiol. 61, 2503–2507.
- Statista: https://de.statista.com/statistik/daten/studie/167671/umfrage/anzahl-der-biogasanlagen-in-deutschland-seit-1992/ (accessed 23.06.2021).
- Strang O, Ács N, Wirth R, Maróti G, Bagi Z, Rákhely G, Kovács KL. 2017. Bioaugmentation of the thermophilic anaerobic biodegradation of cellulose and corn stover. Anaerobe. 46, 104-113.
- Sundberg C, Al-Soud WA, Larsson M, Alm E, Yekta SS, Svensson BH, Sørensen SJ, Karlsson A. 2013. 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. FEMS Microbiol Ecol 85, 612 – 626.
- Strous M, Sharp C. 2018. Designer microbiomes for environmental, energy and health biotechnology. Curr Opin Microbiol. 43, 117-123.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Tada C, Tsukahara K, Sawayama S. 2006. Illumination enhances methane production from thermophilic anaerobic digestion. Appl. Microbiol. Biotechnol. 71, 363–368.
- Tang J, Zhuang L, Ma J, Tang Z, Yu Z, Zhou S. 2016. Secondary Mineralization of Ferrihydrite Affects Microbia Methanogenesis in *Geobacter – Methanosarcina* Cocultures. Applied and Environ Microbiol. 82, 5869 – 5877.
- Tenenbaum DJ. 2008. Food vs. Fuel: Diversion of Crops Could Cause More Hunger. Environ Health Perspect. 116, A254-A257.
- Thompson IP, Van der Gast CJ, Ciric L, Singer AC. 2005. Bioaugmentation for bioremediation: the challenge of strain selection. Environ Microbiol. 7, 909-15.
- Wainaina S, Mukesh Kumar Awasthi L, Taherzadeh MJ. 2019. Bioengineering of anaerobic digestion for volatile fatty acids, hydrogen or methane production: A critical review. Bioengineered. 10, 437–458.
- Town JR, Dumonceaux J. 2015. Laboratory-scale bioaugmentation relieves acetate accumulation and stimulates methane production in stalled anaerobic digesters. Appl Microbiol Biotechnol. 100, 1009 – 1017.
- Vanwonterghem I, Evans PN, Parks DH, Jensen PD, Woodcroft BJ, Hugenholtz P, Tyson GW. 2016. Methylotrophic methanogenesis discovered in the archaeal phylum Verstraetearchaeota. Nature Microbiol. 1, 16170.
- Wang J, Yin Y. 2018. Fermentative hydrogen production using pretreated microalgal biomass as feedstock. Microb Cell Fact. 17:22.
- Wan J, Jing Y, Rao Y, Zhang S, Luo G. 2018. Thermophilic Alkaline Fermentation Followed by Mesophilic Anaerobic Digestion for Efficient Hydrogen and Methane Production from Waste-Activated Sludge: Dynamics of Bacterial Pathogens as Revealed by the Combination of Metagenomic and Quantitative PCR Analyses. Appl Environ Microbiol. 84:e02632-17.
- Weiland P. 2005. Landwirtschaftliche Vergärungsanlagen, In: Anaerobtechnik, ed. Bischofsberger, W., Dichtl, N., Rosenwinkel, K.H., Seyfried, C.F., Böhnke, B. (Springer) ISBN 3-540-06850-3.
- Weiland P. 2009. Biogas production: Current state and perspectives. Appl Microbiol Biotechnol. 85, 849–860.
- Whipps J, Lewis K, Cooke R. Mycoparasitism and plant disease control. In: Burge M, editor. Fungi Biol Control Syst. Manchester University Press; 1988. p. 161-187.
- Xia A, Murphy JD. 2016. Microalgal Cultivation in Treating Liquid Digestate from Biogas Systems. Trends Biotechnol. 34, 265-275.
- Xu S, Han R, Zhang Y, He C, Liu H. 2018. Differentiated stimulating effects of activated carbon on methanogenic degradation of acetate, propionate and butyrate. Waste Manag. 76, 394–403.
- Yan M, Treu L, Zhu X, Tian H, Basile A, Fotidis IA, Campanaro S, Angelidaki I. 2020. Insights into Ammonia Adaptation and Methanogenic Precursor Oxidation by Genome-Centric Analysis. Environ Sci Technol. 54:19.
- Yanuka-Golub K, Baransi-Karkaby K, Szczupak A, Reshef L, Rishpon J, Shechter R, Gophna U, Sabbah I. 2019. An electrode-assisted anaerobic digestion process for the production of high-quality biogas. Water Sci Technol. 79, 2145-2155.
- Ye J, Hu A, Ren G, Zhou T, Zhang G, Zhou S. 2018. Red mud enhances methanogenesis with the simultaneous improvement of hydrolysis-acidification and electrical conductivity. Bioresour Technol. 247, 131 – 137.
- Yenigün O, Demirel B. 2013. Ammonia inhibition in anaerobic digestion: A review. Process Biochemistry 48, 901-911.
- Yurkov VV, Beatty JT. 1998. Aerobic Anoxygenic Phototrophic Bacteria. Microbiol Mol Biol Rev. 62, 695-724.

This project has been granted funding from the European Union's Horizon 2020 research and innovation programme

- Zhang P, Shen Y, Guo JS, Li C, Wang H, Chen YP, Yan P, Yang JX, Fang F. 2015. Extracellular protein analysis of activated sludge and their functions in wastewater treatment plant by shotgun proteomics. Sci Rep. 5:12041.
- Zhang M, Ma Y, Ji D, Li X, Zhang J, Zang L. 2019. Synergetic promotion of direct interspecies electron transfer for syntrophic metabolism of propionate and butyrate with graphite felt in anaerobic digestion. Bioresour Technol. 287, 121373.
- Zheng Y, Harris DF, Yu Z, Fu Y, Poudel S, Ledbetter RN, Fixen KR, Yang ZY, Boyd ES, Lidstrom ME, Seefeldt LC, Harwood CS. 2018. A pathway for biological methane production using bacterial iron-only nitrogenase. Nat. Microbiol. 3, 281.
- Ziels RM, Svensson BH, Sundberg C, Larsson M, Karlsson A, Yekta S. 2018. Microbial rRNA gene expression and co-occurrence profiles associate with biokinetics and elemental composition in full-scale anaerobic digesters. 11, 694-709

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7. New technologies and innovations

Authors: Asier Ortega, Adriel Latorre, Kristie Tanner

8. Case of study of a public initiative: Aras de los Olmos

Author: Mutaz Alajami (Sersuma SLU, Aras de los Olmos)

8.1 Town description

8.1.1 Features

Aras de los Olmos (**Figure 81**) is a town in the Serranía of the province of Valencia, in the heart of the Alto Turia. Located 98 kilometers away from the capital (Valencia City) at altitude of 936 meters above the sea level. It has a continental Mediterranean climate, with cold winters and very mild summers. Snow is usually seen several times a year. Aras is located in the northwest of the province. Between two rivers Turia and Arcos. As most of mountainous villages it has an irregular surface. It has some plains in the central part. But the mountainous areas predominate, with some very deep ravines. It has heights that exceed 1,300 meters of altitude. In the same municipality, about 5 km from the town of Aras there is a very small village called Losilla, where you can see the fall of the river Arcos, the "Sierra del Javalambre" and the old mill of the Central. Aras has a population of 374 inhabitants in 2021. During the summer season it is well over 1.000 inhabitants. The main economical activities based on livestock and agriculture.

8.1.2 Grid

The grid in the municipality belonge to SERSUMA ("Servicios y Suministros Municipales Aras, SL", a public entity dependent on the Ayuntamiento de Aras de los Olmos supplies Losilla and Aras de los Olmos with electric power. The approximate length of the electric line of 20 kV is 11.9 km with two parts, the first from El Collado (Village belongs to Alpuente municipality) to Losillas (5.2 km) and the second to Aras with approximately 6.7 km. Occasionally there is some connection to dispersed customers (mainly farms, some with photovoltaic production). Most consumption it is basically in the town of Aras (90% of the total), and about 10% in Losilla. The high voltage distruption line is a 20 kV with LA56 conductors (current denomination 47-AL1/8-ST1A). Most part of this line cross the mountainous orography in a zone with a lot of storms and wind. All that makes electricity supply to Aras not god enough for XXI century, with a high number of interruptions also with problems of unstable voltage and the continuity of service.

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Figure 70: Arras de los Olmos.

8.2 Situation of Aras de los Olmos

8.2.1 Covenant of mayors

Following the adoption in 2008 of the EU Climate and Energy Package until 2020, the European Commission launched the Covenant of Mayors to support local authorities' efforts to implement sustainable energy policies.
Based on the success of the Covenant of Mayors, the Mayors Adapt initiative was launched in 2014, based on the same governance model. Through which cities were invited to make political commitments and take action to anticipate the inevitable effects of climate change.
At the end of 2015, the two initiatives were merged into the new Covenant of Mayors for Climate and Energy, which embraced the EU's 2030 targets and adopted a comprehensive approach to climate change mitigation and adaptation.
The signatories support a common vision for the year 2050: to accelerate the decarbonization of their territories, strengthen their capacity to adapt to the inevitable effects of climate change and enable their populations to have access to safe, sustainable and affordable energy sources.
Aras de los Olmos is a signatory town of the pact, showing its commitment to the environment and future generations. Commitment to its territory and its people.

8.2.2 SERSUMA SL

SERSUMA SL (**Figure 82**), in view of the problems with the electricity supplies described in the previous point, intends to develop with the ayuntamiento of Aras de los Olmos a project for the use of renewable energies and energy efficiency in the town of Aras de los Olmos. For which, a series of actions are initiated, led by the Ayuntamiento Aras de los Olmos, to

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achieve the environmental objectives of the town and solve the problems of the electric service.

Supply problems, which mean the lack of electricity in the municipality, even for consecutive days during storms. Problems that negatively affect the residents as a whole and, in particular, local businesses.

Electricity supply and internet connection is a fundamental element for rural development and the maintenance of life in the villages. Therefore, it is of vital importance to solve this problem in order to avoid depopulation and promote rural growth.

Figure 71: Logo of Sersuma SLU.

8.3 Renewable Energy Implementation Center

8.3.1 Actions

As a consequence of the above points, and with the objective of Ayuntamiento of Aras de los Olmos to develop a project for the use of renewable energies and energy efficiency. The latter, commissioned, in 2016, the GEDER group, Department of Electrical Engineering, the realization of a Viability Study for the Integration of Sustainable Energy Resources in Aras de los Olmos. In this way, the processing of the Renewable Energy Implementation Center in Aras de los Olmos begins. Subsequently, the Ayuntamiento of Aras de los Olmos, commissioned GEDER group, the drafting of all the preliminary projects of the installations. They include four plants for the production of electrical energy, one from wind power, another photovoltaic plant, a hydroelectric plant and a biomass plant. In June 2017, it was submitted to the Department of Agriculture, Rural Development, Climate Emergency and Ecological Transition (of the Comunidad Valenciana), Start Request for the processing of a Special Plan for the Implementation of Renewable Energies in the town of Aras de los Olmos. The Request for Initiation consisted of the Initial Strategic Document and the Draft of the Special Plan for Renewable Energies of Aras de los Olmos. In February 2018, the GEDER UPV group carried out the Environmental Impact Assessment. It consists of determining the direct or indirect, significant effects of the project on the environment and allow to take appropriate decisions to prevent and minimize such effects. On February 28, 2019, the Environmental Assessment Commission issued a Scoping Document for the Strategic Environmental and Territorial Study. In the document, the considerations to be established in the preparation of the Strategic Environmental and Territorial Study and the Preliminary version of the Special Plan are included. Following this, Aras de los Olmos continued the procedure through the preparation

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of the Preliminary Version of the Special Plan and the preparation of the Strategic Environmental and Territorial Study.

With the Special Renewable Energy Plan of Aras de los Olmos, it is proposed to replace the current energy supply of Aras de los Olmos and its village of Losilla by a combined supply of four renewable energies. Wind turbines, solar panels, hydropower and biogas, which between all of them represent the self-generation of the total consumption of the town of Aras de los Olmos. The different energies, are exposed in the following point. Through these procedures, the aim is to make the implementation of a Renewable Energy Center environmentally and urbanistically viable. The aim is to present it to European instances in order to access financing instruments as signatories of the Covenant of Mayors for Climate and Energy. Mention should be made of the Landscape Integration Study, which is part of the documentation accompanying the Special Plan for the Implementation of Renewable Energies in the town of Aras de los Olmos. The objective of the Study is to analyze and evaluate the landscape impact of the installations necessary for the implementation of a Renewable Energy System.

By means of the Special Plan for the Implementation of Renewable Energies and its mandatory Strategic Environmental and Territorial Study, it includes, among other things, a modification of its Urban Development Regulations. In order to reinforce, make explicit and make possible the achievement of the objectives pursued. This means that, at the same time and through practically the same procedures, the General Structural Urban Development Plan of Aras de los Olmos has been carried out. The Plan is intended to abandon the criteria of urban growth, based on the occupation of new territories, to focus on sustainable growth using all existing resources in urban land. This Sustainable Model can be culminated with the implementation of a renewable energy system that would satisfy the energy needs of the town. To this end, the Special Plan for the Implementation of Renewable Energies is being processed. It should be noted that on December 10, 2015 the Wildlife Service reports that the proposed General Structural Plan will not have appreciable effects on the Natura 2000 Network. Consequently, it is considered that the project should not be subject to detailed assessment of its effects on the Natura 2000 Network. The Ayuntamiento of Aras de los Olmos, made all the documentation expressed, available for public participation and consultation. As legally required.
In this regard, on June 12, 2020, the Plenary of the Ayuntamiento initially approved the Plan. On June 29, 2020, the approval was published in the "Diari Oficial Generalitat Valenciana" No. 8845 in order to initiate the 45 days of public information, as required by the regulations. It is currently in the process of being processed. It should be taken into account that the probable evolution in the case of non-application of the Special Plan is obvious, it means an increase of CO2 emissions to the atmosphere. Equivalent to the consumption of the entire population of right and floating in holiday periods. This is a negative implication from the perspective of combating climate change.

In conclusion, this is a pilot experience in the peninsular part of Spain, to achieve a zero energy balance in a municipality. We understand that the effects of the implementation of

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this project are minimized with corrective measures and are amply compensated by the benefits for the environment in general and for climate change in particular. (A reduction of emissions of approximately 1,200T.CO2/year is estimated). Through the combination of a set of renewable energy systems. Such as wind, solar, hydro and biogas. It is intended a permanent supply that meets the energy needs of the municipality. Although in our case it is a municipality with a very large municipal area and a very small population, this project is developed with the intention of becoming a model to be followed in other places and generalize the use of renewable energies at all levels.

8.3.2 Installations

To achieve the objectives set out above. Based on the Special Plan for the Implementation of Renewable Energies in Aras de los Olmos and the available natural resources. The necessary installations would be the following:

Photovoltaic farm: Solar energy seems to be the most suitable to produce most of the energy needed. It is more stable than wind power. Therefore, the installation of a 700 kW photovoltaic park. It will provide, depending on the solar radiation, most of the energy required, with the following characteristics: 1,680 bifacial photovoltaic solar panels made of monocrystalline material, each with 450 Wp of power. The panels will be placed on a structure with single-axis solar tracking. 6 inverters of 110 kW and one of 40 kW, which will be connected to a 20kV/400V step-up transformer. As it is also planned to supply the plant off-grid, the inverters will have an adjustable power factor and will be able to supply up to a maximum of 700 kW.It needs a 14,000 m2 of occupied area. A newly constructed 20kV line will be provided from the solar power plant to the connection with an existing line of Sersuma.

Wind farm: A wind farm consisting of two 100 kW wind turbines. It is a three-bladed machine, synchronous generator, with multiplier, pitch angle control system and variable speed operation. Its base will house the transformation center for connection to a 20 kV line.

Micro Hydroelectric Power Plant: Another resource that has been contemplated for the Aras energy complex is the hydraulic one, from the Arcos river. This is a small permanent watercourse that flows through a deep ravine next to Losilla. A micro hydroelectric power plant that will serve both as an energy storage and generation element, with a turbination power of 200 kW and a pumping power of 180 kW, which will have: An upper asphalt concrete reservoir, raft type, with a surface area of 8,100 m2 and a storage capacity of 24,000 m^3 of water. It has 48 hours of turbination. A lower reinforced concrete tank to store 4,000 m^3 of water, allowing 9.6 hours of turbination. Two Pelton type turbines of 100 kW hydraulic power each will be used. Two synchronous generators. Suitable to operate both in parallel with the grid and in island, allowing the control of reactive power delivered, voltage regulation and synchronization with the grid. Also there is three 60 kW vertical centrifugal pumps for pumped storage, they will be connected to 20/0.4 kV transformation center for connect the installation to the 20 kV line.

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Biogas plant: For technical purposes, solar and wind production is not controllable and introduces strong and rapid variations in the power generated, which could destabilize the local electricity system. To stabilize the system, it is necessary to have another source such as biogas, which offers the possibility of controllable generation. In contrast to solar and wind energy, it is an attractive option and a very important element of the system.
Therefore, a 200 kW biogas plant is contemplated, which will serve as base generation and to cover the demand at times when there is a lack of other renewable resources, consisting of:

- A 93 m3 collection basin for 3 days of raw material storage.
- A 925.15 m3 digester, which will allow a daily production of 977 m3 of biogas. It will be covered by a double-layer elastic membrane that will allow storing the biogas from 12 hours of production.
- A desulfurizer and a dryer that will allow the use of biogas in a 200 kW internal combustion engine, which will drive the synchronous generator that will generate electricity.

Smart Grid: The energy project of Aras, with the four generation facilities described above, will constitute a micro-grid that will require proper management and control. In order to achieve a system with the capacity to operate autonomously, with the possibility of disconnecting from the grid, it will be necessary to satisfy the total energy demand of the municipality and manage the system efficiently and reliably. For which it is necessary to develop a complex control system that allows the operation of such electrical network as a smart grid.

Some of the key aspects of the development of this control system will be:

- Difficulty in predicting demand, due to the diversity of consumption.
- Difficulty in predicting actual generation capacity, due to the uncertainties involved in the expected generation systems.
- The need for a permanently available, manageable base generator to ensure stable operation in the event of disconnection from the grid.
- Need for a large energy storage system.

As a consequence of the above, the need arises to develop a complex control system, with an automatic primary level and a secondary control from the control center, turning the microgrid into a smart grid.

Micro4Biogas: The participation of the Ayuntamiento of Aras de los Olmos in the project is fundamental for the development of one of the manageable energies, basic in the Plan. The energy is obtained at will, unlike wind and solar. With the intervention of Aras in the project, it will be the protagonist of a pioneering trial in the production of renewable energy, with bacteria optimized for the biodigestion of waste. The scientific strategy will be implemented by MICRO4BIOGAS, with the objective of improving knowledge about biogas production. It will also set a precedent and an example to be followed by other countries. The intervention in the project also represents a possible solution to the problem of the farming community, with regard to the management of slurry and compliance with the European Commission's

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regulations. In Aras de los Olmos there are pig, rabbit and poultry farms. This model allows waste treatment in a controlled manner, avoiding the major impact of natural decomposition. After the biogas production process is completed, the waste can also be reused as agro-ecological fertilizer and the excess water from the process will be used for irrigation. For all these reasons, this project can be considered as a benchmark for the circular economy and, therefore, for rural development. In the previous points, it is necessary to highlight the need of financing for each of the mentioned installations, therefore, parallel to point 2 of this document, administrative actions have been carried out in order to carry out the future construction of the installations.

8.4 Relationship with the different organizations

European organisations: The Ayuntamiento has a relationship with the European Commission, as a funding organization. It is the European organ that has provided the Ayuntamiento with a budget for the implementation of the MICRO4BIOGAS project. It is also behind other actions, since it has provided different Ministries with budget, from which the Ayuntamiento of Aras de los Olmos has benefited through the application for grants and subsidies. The Ayuntamiento has a relationship with several universities and European entities as sources of research in the MICRO4BIOGAS project and derived from the participation in it.

State organizations: The role of the Ministry for Ecological Transition and the Demographic Challenge, hereinafter MITECO, as the organization that provides the Ayuntamiento with financing instruments, should be highlighted. Among other financing, the construction of the biogas plant is co-financed by MITECO and by the General Directorate for Ecological Transition of the Generalitat Valenciana. This last autonomic organism. In the same line as the previous one, we find the IDAE: Institute for Energy Diversification and Saving, as a body attached to the MITECO. This body intervenes by providing funding for projects of technological innovation and replicable nature. It participates in the financing of the Aras energy project, through the line of aid for singular projects of local entities that favor the transition to a low-carbon economy. The Ministry of Territorial Policy and Public Function, in charge of the relations between the Government of the Nation, the Autonomous Communities and the entities that integrate the Local Administration, in our particular case, between the Government of Spain, the Valencian Community and the City Council of Aras de los Olmos.

Autonomous community organizations: At the regional level, mention must be made of the "Conselleria de Agricultura, Cambio Climático", "Medio Ambiente y Desarrollo Rural, through the Direcció General de Medi Natural i d' Avaluació Ambiental", and the "Conselleria de Vivienda, Obras Públicas y Vertebración del Territorio". Organisms to which belongs the processing of the Environmental and Strategic Evaluation, necessary for the Approval of a Special Plan of Renewable Energies in Aras de los Olmos. Also to the "Conselleria de Economía, Industria, Turismo y Ocupación-CONSELLERIA D'ECONOMIA SOSTENIBLE, SECTORS PRODUCTIUS, COMERÇ I TREBALL". In charge of granting administrative authorization for the installation of a transformation center. It has been required to go to these bodies for

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the fulfillment of requirements and formalities necessary for the achievement of the objective of the Ayuntamiento of Aras de los Olmos. The "Diputación de Valencia", in particular the Area of Environment and the Area of Roads, as the entity to whom the roads in the municipality of Aras de los Olmos belong. All this to the extent that these roads could be affected by the project. The adjoining Ayuntamientos, with relevance to the Ayuntamiento of Arcos de las Salinas as far as the hydraulic plant is concerned. Relationship, through consultations, with national or regional companies supplying water, electricity, gas, telephony and telecommunications. For the issuance of reports on the needs and minimum essential technical conditions of the projects, works and installations that, where appropriate, must be executed. Special mention must be made to the Polytechnic University of Valencia, hereinafter UPV, as a public body fully adhered to the project of the Ayuntamiento of Aras de los Olmos. A collaborating and research body that has been part of the project from the beginning. It has elaborated different documents such as the feasibility plan and the different preliminary projects.

Legal regulation: The Ayuntamiento of Aras de los Olmos, for the processing of the renewable energy project, has had to comply with different regulations, both European, national and regional.

European legislation

At the European level, it is worth mentioning the following applicable regulations:

- Directiva 2001/42/CE del Parlamento Europeo y del Consejo de 27 de junio, para la integración de los aspectos ambientales en la toma de decisiones de planes y programas públicos. Dicha Directiva se incorpora al derecho interno español mediante la Ley 21/2013, de 9 de diciembre, de Evaluación Ambiental
- Directiva 2007/60/CE, relativa a la evaluación y gestión de los riesgos de inundación, y su transposición a la legislación estatal mediante el Real Decreto 903/2010, de 9 de julio
- Directiva 92/43/CEE del Consejo, de 21 de mayo de 1992, relativa a la conservación de los hábitats naturales y de la fauna y flora silvestres.
- Directiva 79/409/CEE relativa a la conservación de las aves silvestres.
- Directiva 97/62/CE de 27 de octubre, que modifica los Anexos I y II de la Directiva de Hábitats.
- Reglamento (CE) nº 1069/2009 del Parlamento Europeo y del Consejo de 21 de octubre de 2009 por el que se establecen las normas sanitarias aplicables a los subproductos animales y los productos derivados no destinados al consumo humano
- Directiva 2008/98/CE, del Parlamento Europeo y del Consejo de 19 de noviembre de 2008 sobre los residuos.
- Directiva 99/31/CE del Consejo de 26 de abril 1999 relativa al vertido de residuos.
- Directiva 2008/1/CE del Parlamento Europeo y del Consejo, de 15 de enero de 2008, relativa a la prevención y al control integrado de la contaminación.
- Reglamento (CE) nº 74/2009 del Consejo, de 19 de enero de 2009, por el que se modifica el Reglamento (CE) nº 1698/2005, relativo a la ayuda al desarrollo rural a través del Fondo Europeo Agrícola de Desarrollo Rural.

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- Directiva 2009/28/CE del Parlamento Europeo y del Consejo relativa al fomento del uso de energía procedente de fuentes renovables por la que se modifican y derogan las Directivas 2001/77/CE y 2003/30/CE.
- Directiva 2000/54/CE del Parlamento Europeo y del Consejo, de 18 de septiembre de 2000, sobre la protección de los trabajadores contra los riesgos relacionados con la exposición a agentes biológicos durante el trabajo

State legislation

Spanish law, applicable in the different areas described above (environmental, energy, fauna, flora, atmosphere, etc.), consists of the following legal instruments:

- Ley 21/2013, de 9 de diciembre, de Evaluación Ambiental.
- Ley 24/2013 de 26 de diciembre, del Sector Eléctrico
- Real Decreto Legislativo 7/2015, de 30 de octubre, por el que se aprueba el texto refundido de la Ley de Suelo y Rehabilitación Urbana, en lo que afecta a la evaluación ambiental.
- Real Decreto 903/2010, de 9 de julio, de Evaluación y Gestión de Riesgos de Inundación.
- Texto Refundido de la Ley de Aguas (RDL 1/2001)
- Ley 7/2002, de 3 de diciembre, de Protección contra la Contaminación acústica.
- Normas de Gestión de Espacios Red Natura 2000
- Ley 42/2007, de 13 de diciembre, del Patrimonio Natural y de la Biodiversidad.
- Ley 25/2009, de modificación de diversas leyes para su adaptación a la ley sobre el libre acceso a las actividades de servicios y su ejercicio.
- Real Decreto 1110/2007, por el que se aprueba el Reglamento unificado de puntos de medida del sistema eléctrico.
- Real Decreto 2019/1997, por el que se organiza y regula el mercado de producción de energía eléctrica.
- Orden de 29.12.97, por la que se desarrollan algunos aspectos del Real Decreto 2019/1997, por el que se organiza y regula el mercado de producción de energía eléctrica.
- Orden de 17.12.98 por la que se modifica la Orden de 29.12.97, que desarrolla algunos aspectos del Real Decreto 2019/1997, por el que se organiza y regula el mercado de producción de energía eléctrica.
- Real Decreto 413/2014, de 6 de junio, por el que se regula la actividad de producción de energía eléctrica a partir de fuentes de energía renovables, cogeneración y residuos.
- Orden IET/1045/2014, de 16 de junio, por la que se aprueban los parámetros retributivos de las instalaciones tipo aplicables a determinadas instalaciones de producción de energía eléctrica a partir de fuentes de energía renovables, cogeneración y residuos.
- Real Decreto 661/2007, de 25 de mayo, por el que se regula la actividad de producción de energía eléctrica en régimen especial, derogado por el Real Decreto 413/2014.
- Corrección de errores del Real Decreto 661/2007, de 25 de mayo, por el que se regula la actividad de producción de energía eléctrica en régimen especial. (BOE de 25 de julio de 2007).
- Corrección de errores del Real Decreto 661/2007, de 25 de mayo, por el que se regula la actividad de producción de energía eléctrica en régimen especial (BOE de 26 de julio de 2007).
- Real Decreto 1565/2010, de 19 de noviembre, por el que se regulan y modifican determinados aspectos relativos a la actividad de producción de energía eléctrica en régimen especial.
- Real Decreto 1614/2010, de 7 de diciembre, por el que se regulan y modifican determinados aspectos relativos a la actividad de producción de energía eléctrica a partir de tecnologías solar termoeléctrica y eólica.

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- Real Decreto-Ley 14/2010, de 23 de diciembre, por el que se establecen medidas urgentes para la corrección del déficit tarifario del sector eléctrico.
- Real Decreto 1544/2011, de 31 de octubre, por el que se establecen los peajes de acceso a las redes de transporte y distribución que deben satisfacer los productores de energía eléctrica.
- Real Decreto-Ley 6/2009, de 30 de abril, por el que se adoptan determinadas medidas en el sector energético y se aprueba el bono social.
- Resolución de 19 de noviembre de 2009, de la Secretaría de Estado de Energía, por la que se publica el Acuerdo del Consejo de Ministros de 13 de noviembre de 2009, por el que se procede a la ordenación de los proyectos o instalaciones presentados al registro administrativo de preasignación de retribución para las instalaciones de producción de energía eléctrica, previsto en el Real Decreto Ley 6/2009.
- Real Decreto 1955/2000, por el que se regulan las actividades de transporte, distribución, comercialización, suministro y procedimientos de autorización de instalaciones de energía eléctrica.
- Real Decreto 1454/2005, de 2 de diciembre, por el que se modifican determinadas disposiciones relativas al sector eléctrico.
- Orden ITC/3519/2009, de 28 de diciembre, por la que se revisan los peajes de acceso a partir del 1 de enero de 2010 y las tarifas y primas de las instalaciones de régimen especial.
- Real Decreto 1028/2007, de 20 de julio, por el que se establece el procedimiento administrativo para la tramitación de las solicitudes de autorización de instalaciones de generación de energía eléctrica en el mar territorial.
- Real Decreto 198/2010, de 26 de febrero, por el que se adaptan determinadas disposiciones relativas al sector eléctrico a lo dispuesto en la Ley 25/2009, de modificación de diversas leyes para su adaptación a la ley sobre el libre acceso a las actividades de servicios y su ejercicio.
- Corrección de errores del Real Decreto 198/2010, de 26 de febrero, por el que se adaptan determinadas disposiciones relativas al sector eléctrico a lo dispuesto en la Ley 25/2009, de modificación de diversas leyes para su adaptación a la ley sobre el libre acceso a las actividades de servicios y su ejercicio.
- Real Decreto 1699/2011, de 18 de noviembre, por el que se regula la conexión a red de instalaciones de producción de energía eléctrica de pequeña potencia.
- Real Decreto-ley 1/2012, de 27 de enero, por el que se procede a la suspensión de los procedimientos de preasignación de retribución y a la supresión de los incentivos económicos para nuevas instalaciones de producción de energía eléctrica a partir de cogeneración, fuentes de energía renovables y residuos.
- Real Decreto-ley 20/2012, de 13 de julio, de medidas para garantizar la estabilidad presupuestaria y de fomento de la competitividad. Título VII, Medidas para la supresión de desajustes entre los costes e ingresos en el sector eléctrico.
- Orden IET/1168/2014, de 3 de julio, por la que se determina la fecha de inscripción automática de determinadas instalaciones en el registro de régimen retributivo específico previsto en el título V del Real Decreto 413/2014.
- Orden IET/1345/2015, de 2 de julio, por la que se establece la metodología de actualización de la retribución a la operación de las instalaciones don régimen retributivo específico.
- Ley 16/2002, de 1 de julio de prevención y control integrados de la contaminación.
- Ley 39/2015, de 1 de octubre, del Procedimiento Administrativo Común de las Administraciones Públicas, por ser una entidad local, Ayuntamiento.
- Decreto 27/2007 de Responsabilidad Medioambiental.
- Decreto 27/2006, por la que se regulan los derechos de acceso a la información, de participación pública y de acceso a la justicia en materia de medio ambiente.
- Real Decreto 139/2011, de 4 de febrero, para el desarrollo del Listado de Especies Silvestres en Régimen de Protección Especial y del Catálogo Español de Especies Amenazadas.

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- Corrección de errores del Real Decreto 139/2011, de 4 de febrero, para el desarrollo del Listado de Especies Silvestres en Régimen de Protección Especial y del Catálogo Español de Especies Amenazadas
- Real Decreto 1997/1995, de 7 de diciembre, que establece medidas para contribuir a garantizar la biodiversidad mediante la conservación de la flora y la fauna silvestres y de sus hábitats naturales. Transpone la Directiva 92/43/CEE al ordenamiento jurídico español.
- Real Decreto 1193/1998, de 12 de junio, por el que se modifica el Real Decreto 1997/1995, de 7 de diciembre, que establece medidas para contribuir a garantizar la biodiversidad mediante la conservación de la flora y la fauna silvestres y de sus hábitats naturales. Transpone la Directiva 92/43/CEE al ordenamiento jurídico español.
- Ley 37/2003, de 17 de noviembre, de Ruido.
- Real Decreto 1367/2007, de 19 de octubre, por el que se desarrolla la Ley 37/2003, de 17 de noviembre, del Ruido, en lo referente a la zonificación acústica, objetivos de calidad y emisiones acústicas.
- Ley 41/1997, de 5 de noviembre, por la que se modifica la Ley 4/1989, de 27 de marzo, de Conservación de Espacios Naturales y de la Flora y Fauna Silvestres.
- Ley 3/1995, de 23 de marzo, de Vías Pecuarias.
- Ley 21/2015, de 20 de julio, por la que se modifica la Ley 43/2003, de 21 de noviembre, de Montes.
- Ley 21/1992, de 16 de julio, de Industria.
- Real Decreto 223/2008, de 15 de febrero, por el que se aprueban el Reglamento sobre condiciones técnicas y garantías de seguridad en líneas eléctricas de alta tensión y sus instrucciones técnicas complementarias ITC-LAT 01 a 09.
- Real Decreto 337/2014, de 9 de mayo, por el que se aprueban el Reglamento sobre condiciones técnicas y garantías de seguridad en instalaciones eléctricas de alta tensión y sus instrucciones técnicas complementarias.
- Real Decreto 842/2002, de 2 de agosto, por el que se aprueba el Reglamento Electrotécnico para baja tensión.
- Decreto 2414/1961, de 30 de noviembre, por el que se aprueba el Reglamento de actividades molestas, insalubres, nocivas y peligrosas.
- Real Decreto Legislativo 2/2004, de 5 de marzo, por el que se aprueba el texto refundido de la Ley Reguladora de las Haciendas Locales.
- Ley 58/2003, de 17 de diciembre, General Tributaria.
- Ley 38/1992, de 28 de diciembre, de Impuestos Especiales
- Real Decreto 1165/1995, de 7 de julio de 1995, por el que se aprueba el Reglamento de los Impuestos Especiales.
- Ley 66/1997, de 30 de diciembre, de Medidas Fiscales, Administrativas y del Orden Social (crea el Impuesto sobre la Electricidad).
- Real Decreto 112/1198, de 30 de enero, por el que se modifica el Reglamento de los Impuestos Especiales
- Real Decreto 1247/2008 de 18 de julio, Instrucción de Hormigón Estructural, EHE-08
- Real Decreto 314/2006, Código Técnico de la Edificación
- Real Decreto 1429/2003. SANDACH
- Ley 10/1998, de 21 de abril, de Residuos.
- Orden MAM/304/2002, de 8 de febrero, por la que se publican las operaciones de valorización y eliminación de residuos y la lista europea de residuos.
- Orden ITC/1522/2007, de 24 de mayo, por la que se establece la regulación de la garantía del origen de la electricidad procedente de fuentes de energía renovables y de cogeneración de alta eficiencia.

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Autonomous community legislation

At the autonomous community level, as a municipality belonging to the Valencian Community, it is important to highlight the following regulations applicable to the matters described above:

- Ley 1/2019, de 5 de febrero, de modificación de la Ley 5/2014, de 25 de julio, de Ordenación del Territorio, Urbanismo y Paisaje de la Comunitat Valenciana.
- Decreto 1/2011 de 13 de enero del Consell por el que se aprueba la Estrategia Territorial de la Comunitat Valenciana y el Decreto 166/2011 de 4 de noviembre del Consell por el que se modifica el Decreto 1/2011 de 13 de enero del Consell por el que se aprobó la Estrategia Territorial de la Comunitat Valenciana (ETCV). Para el correcto desarrollo del proyecto se han seguido las directrices de la ETCV.
- Decreto 58/2013, de 3 de mayo, del Consell, por el que se aprueba el Plan de Acción Territorial Forestal de la Comunitat Valenciana (PATFOR)
- Orden 22/2012, de 13 de noviembre, de la Consellería de Infraestructuras, Territorio y Medio Ambiente, por la que se publica el Catálogo de árboles monumentales y singulares de la Comunitat Valenciana
- Ley 3/2014, de 11 de julio, de la Generalitat, de Vías Pecuarias de la Comunitat Valenciana y la Orden de 23 de noviembre de 1988, de la Consellería de Agricultura y Pesca, por la que se aprueba la Clasificación de las Vías Pecuarias
- Plan de Acción Territorial sobre Prevención del Riesgo de Inundación en la Comunitat Valenciana (PATRICOVA) aprobado por Decreto 201/2015, de 29 de octubre del Consell.
- Decreto 104/2006, de 14 de julio, del Consell, de Planificación y Gestión en materia de Contaminación Acústica.
- Decreto 81/2013, de 21 de junio, del Consell, de aprobación definitiva del Plan Integral de Residuos de la Comunitat Valenciana (PIRCV).
- Plan Especial ante el Riesgo de Accidentes en el Transporte de Mercancías Peligrosas por Carretera y Ferrocarril, aprobado por Decreto 49/2011, de 6 de mayo, del Consell.
- Estrategia Valenciana ante el Cambio Climático 2013-2020, aprobada por el Consell de la Generalitat Valenciana en fecha 22 de febrero de 2013; marco de referencia en materia de cambio climático, ya que la normativa sectorial de cambio climático no establece requisitos específicos para los planes de ordenación.
- Plan Eólico de la Comunitat Valenciana. Aprobado por Acuerdo de 26 de julio de 2001, del Gobierno Valenciano.
- Decreto 74/2016, de 10 de junio, del Consell, por el que se aprueba el Reglamento por el que se determina la referenciación cartográfica y los formatos de presentación de los instrumentos de planificación urbanística y territorial de la Comunitat Valenciana.
- Decreto 162/1990, de 15 de octubre, del Consell de la Generalitat Valenciana, por el que se aprueba el Reglamento para la ejecución de la Ley 2/1989, de 3 de marzo, de la Generalitat, de Impacto Ambiental.
- Decreto 32/2006, de 10 de marzo, del Consell de la Generalitat, por el que se modifica el Decreto 162/1990.
- Ley 3/1993 Forestal de la Comunidad Valenciana.
- Decreto 98/1995, de 16 de mayo, del Gobierno Valenciano, por el que se aprueba el Reglamento de la Ley 3/1993, de 9 de diciembre, Forestal de la Comunidad Valenciana.
- Decreto 32/2004, de 27 de febrero, del Consell de la Generalitat, por el que se crea y regula el Catálogo Valenciano de Especies de Fauna Amenazadas y se establecen categorías y normas para su protección.

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- Decreto 70/2009, de 22 de mayo, del Consell, pro el que se crea y regula el Catálogo Valenciano de Especies de Flora Amenazadas y se regulan medidas adicionales de cosnervación. –
- Real Decreto 265/1994, de 20 de diciembre, del Gobierno Valenciano, por el que se crea y regula el Catálogo Valenciano de Especies Amenazadas de Fauna y se establecen categorías y normas de protección de la fauna.
- Orden de 20 de diciembre de 1985, de la Conselleria de Agricultura y Pesca, sobre protección de especies endémicas o amenazadas.
- Orden de 4 de mayo de 1999, de la Conselleria de Medio Ambiente, por la que se declarna 33 microreservas vegetales en la provincia de Alicante y 29 microreservas vegetales en la provincia de Valencia.
- Orden de 6 de noviembre de 2000, de la Conselleria de Medio Ambiente, por la que se declaran 23 microreservas vegetales en la provincia de Valencia.
- Orden de 22 de octubre de 2002, de la Conselleria de Medio Ambiente, por la que se declaran 22 microreservas vegetales en la provincia de Valencia.
- Orden de 17 de julio de 2006, de la Conselleria de Territorio y Vivienda, por la que se declaran 16 microreservas vegetales en la provincia de Valenica.
- Orden de 30 de enero de 2007, de la Conselleria de Territorio y Vivienda, por al que se declara una microreserva vegetal en la provincia de Valencia.
- Resolución de 15 de octubre de 2010, del conceller de Medio Ambiente, Agua, Urbanismo y Vivienda y vicepresidente tercero del Consell, por la que se establecen las zonas de protección de la avitauna contra la colición y electorcución, y se ordenan medidas para la reducción de la mortalidad de aves en líneas eléctricas de alta tensión.
- Ley 26/2007, de 23 de octubre, de Responsabilidad medioambiental.
- Ley 11/1994, de 27 de diciembre, de la Generalitat Valenciana, de Espacios Naturales Protegidos.
- Decreto 60/2012, de 5 de abril del Consell, por el que se regula el régimen especial de evaluación y de aprobación, autorización o conformidad de planes, programas y proyectos que puedan afectar a la Red Natura 2000.
- Acuerdo de 5 de junio de 2009, del Consell, de ampliación de la Red de Zonas de Especial Protección para las Aves (ZEPA) de la Comunitat Valenciana.
- Modificación de la Ley 7/2002, de 3 de diciembre, de la Generalitat, de protección contra la contaminación acústica.
- Instrucción de 13 de enero de 2012, de la Dirección general de Medio Natural, sobre vías pecuarias.
- Ley 10/2000, de 12 de diciembre, de Residuos de la Comunidad Valenciana.

Local legislation: Finally, at the municipal level, mention must be made of the General Urban Development Plan of Aras de los Olmos.

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9. Stakeholders in anaerobic digestion

Author: Pascal Otto (Technische Universität Dresden)

Abstract: The biogas sector is very complex and affects many areas. The complexity of this sector is illustrated in the following **figure 83**. The biogas producers are at the centre of the biogas sector. Around the biogas producers are all the areas that are tangential to them. For simplicity, only the primary relationships of the stakeholders are shown. This means that farmers, for example, are of course also connected to the European Union, but this connection is of secondary importance for the biogas sector itself. The exact relationships of the stakeholders to each other are dealt with in the respective chapter. It will focus on the stakeholders' biogas producers, biogas plant planners, authorities, research and development, laboratories, farmers, electricity, and gas grid owners, energy suppliers, the general public, the national government, and the European Union.

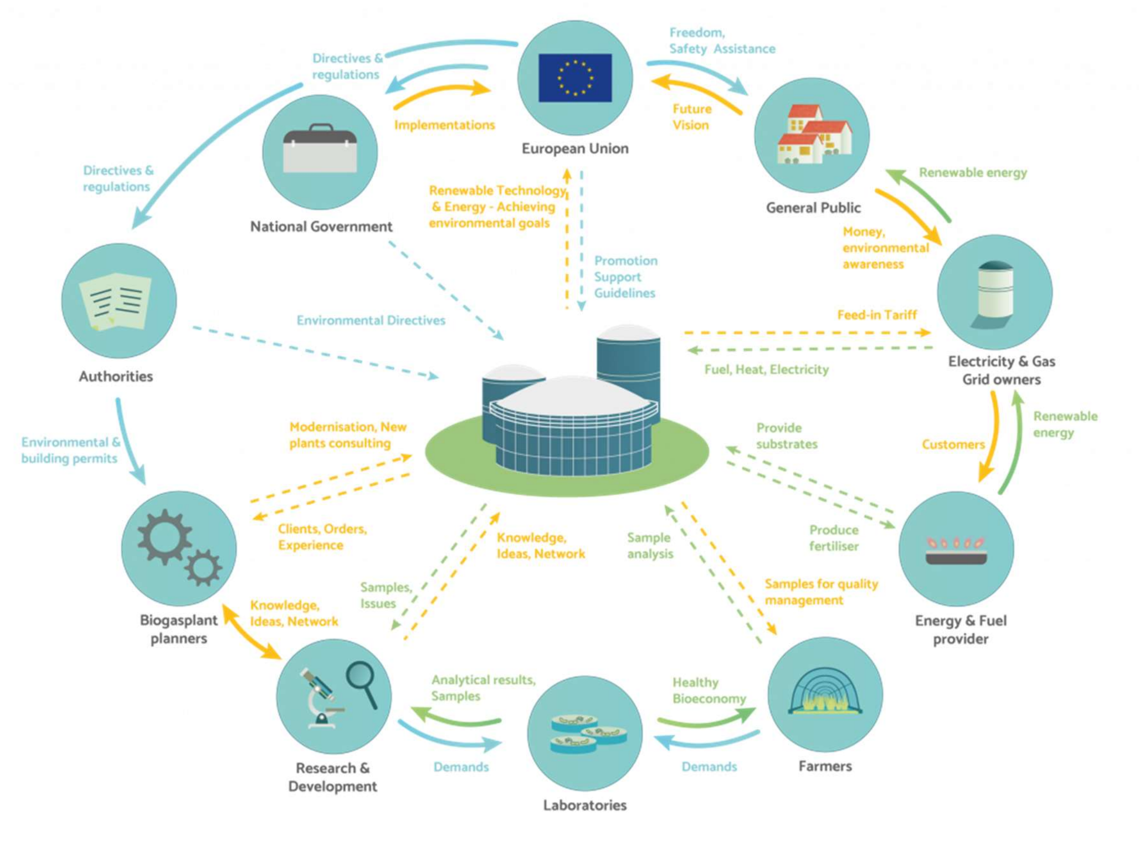

Figure 72: Schematic representation of the biogas sector with biogas producers at the centre and all primary stakeholder relationships explained. Green represents material flows, yellow indicates services and consulting, and blue shows regulations and guidelines.

9.1 Biogas producers

Biogas producers are at the centre of the entire biogas sector and have or have had some form of contact with every stakeholder in the biogas sector. They form the main value chain

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as they ensure that valuable resources such as biogas, bio-fertilisers, bio-methane, heat, electricity are produced from organic materials such as bio-waste, animal excreta, green waste, sewage sludge, energy crops, agricultural waste and food waste. In further explanations, the relationships between the biogas producer and the stakeholders involved are presented.

Biogas producer & Biogas plant planners: Before a biogas or bio-methane plant is built, the potential plant operator is in contact with a biogas plant planner that is to take over the construction and planning. A lot of data must be collected in advance so that the biogas planner can take over the conceptual design and dimensioning of the biogas plant. This includes:

- Quantity of the substrate
- Quality of the substrate
- Composition of the substrate
- Location of the potential biogas plant
- Local circumstances
- Potential uses: heat, electricity or bio-methane.

Subsequently, the biogas plant planner is an important intermediary between the biogas producer and the local government, the authorities, research & development.

The construction of the biogas plant and the feed-in tariff are discussed with the local government, biogas plant planner and the biogas plant operators. This includes the achievement of climate targets and sustainable development and growth.

With the authorities, the impact on the environment and the general construction of the plant are discussed. Therefore, the biogas plant planner and the biogas plant operator work hand in hand to get all documents and formalities. All necessary environmental and building permits must be obtained for the construction of the plant. These ensure that the construction will not adversely affect the surrounding ecosystems, people, buildings and environment. This can take 6 – 24 months depending on the conditions and the planned concepts.

The biogas plant planner is responsible for ensuring that the construction of the biogas plant begins after all necessary permits and approvals have been obtained. For this purpose, a company is contracted to build the plant under the guidance of the biogas plant planner and the biogas plant operator. This can take 1 to 5 years depending on the conditions, the required building materials and possible problems.

After the plant has been built, the biogas plant planner is available as an advisor to the biogas plant operator. Problems with the technology, the anaerobic digestion or other elements of the process can be discussed and solved. In addition, the biogas plant planner informs the biogas plant operator about potential modernisations. In return, the biogas plant operator provides the biogas plant planner with valuable data about the process, the components and the efficiency of the biogas plant.

As already discussed, the biogas plant operator is usually in contact with the authorities, the national or local government and the European Union via the biogas plant planner. The most important aspect is that the European Union, the national governments and the authorities want to achieve the goals of climate neutrality, increasing biodiversity and promoting the circular economy. The construction of biogas plants is a component that contributes to the

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realisation of these goals. Through the implementation of directives, regulations and funding, biogas producers are influenced and supported on a European and national level.

The biogas plant operator is also in contact with the operators of the electricity, heat or gas network, as the produced electricity, heat or gas is fed in at interfaces. The biogas plant operator receives money for feeding in the corresponding resource. There are different remuneration models. These depend on the substrate used, the resource fed into the grid, the size of the plant and the year of construction. The subsidy comes from the national or European government. Subsidies can also be paid in different ways:

- A fixed negotiated tariff is paid per kWh.
- The current feed-in tariff to the electricity, gas or heat network is paid
- The current feed-in tariff to the electricity, gas or heat network is paid plus a predefined flat rate

In case the biogas plant operators are not farmers themselves, they are in contact with the farmers. Farmers provide a variety of organic waste such as green waste, silage, animal excrement and energy crops. This makes them essential for the operation of biogas plants in rural areas. In return, the biogas plant delivers concentrated and very good fertiliser, which is cheaper than synthetic fertiliser and is also recycled through the use of raw materials to create new products.

The relationship between biogas producers and laboratories is relatively simple. The biogas producers deliver samples to the laboratory for analysis of various chemical and microbial laboratory parameters. The laboratories then continuously receive orders and analyse these samples. In doing so, they observe the legally prescribed limit values for the recycled usage of the digestate in agriculture. Summarized the laboratories are important for quality management and monitoring of the biogas plants.

The biogas plant operators are also in contact with research & development. The interfaces between the two actors are multifaceted and range from optimisation of the microbiome, improvement of the plant operation, general validation, etc. Communication can take place in two different ways:

1. The biogas plant operator comes to the research institutions with a specific problem, and the research institutions respond with analyses, ideas, information or establishing contacts with partners
2. The research institutions want to investigate a specific issue and ask the biogas plant operator for samples, information and/or technical data.

The research & development department can also work with any other stakeholder to evaluate and address specific issues.

The general public does not interface directly with the biogas plant operator. They usually only purchase electricity, heat, and/or methane from the biogas plants over the energy providers. However, it is extremely important to educate people about the relevance, effectiveness and circularity of biogas plants. This increases the acceptance of biogas plants among the population and they are seen as a positive development in the expansion of renewable energy sources.

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9.2 Biogas planers

Besides biogas plant operators, biogas plant planners have one of the most important tasks. Through their work, they help to build, expand and modernise biogas plants and consequently promote the biogas landscape. They are an important communication and planning interface between biogas plant operators and all other stakeholders. In the following, the relationships between biogas plant planners and all other stakeholders are presented.

Before a biogas or bio-methane plant is built, the potential biogas plant operator is in contact with a biogas plant planner that is to take over the construction and planning. A lot of data must be collected in advance so that the biogas planner can take over the conceptual design and dimensioning of the biogas plant. This includes:

- Quantity of the substrate
- Quality of the substrate
- Composition of the substrate
- Location of the potential biogas plant
- Local circumstances
- Potential uses: heat, electricity or bio-methane.

With this data, the biogas plant planner drafts an initial concept for a suitable biogas plant for the respective location. The planning takes into account:

- Used substrates
- Available space
- Budget of the plant operator
- Funding systems
- Construction and environmental permits
- Suppliers
- Materials and personnel for the construction of the biogas plant

The construction of the biogas plant and all components involved is organised by the biogas plant planners and carried out in consultation with the biogas plant operator. After the construction of the biogas plant is completed, the biogas plant planner acts as a service provider to the biogas plant operator. The biogas plant planner helps with problems and informs about new subsidy models, modernisation programmes and important renovations that have to be carried out.

In order to ensure the construction of the biogas plant and all other components such as gas storage, combined heat and power plant, secondary fermentation storage, etc., building and environmental permits must be obtained. For this purpose, the biogas plant planners are in contact with the authorities and with companies that are responsible for the certification of all components of a biogas plant. The biogas plant planners present the concept for the construction and operation of the biogas plant in cooperation with the biogas plant operators. Usually, the construction of a biogas plant is not started until the permit has been obtained or at least it is clear how the plant will be approved. After the approval, the biogas plant planner is also given a large number of requirements and handling instructions, including how certain parts of the plant must be built or should function. For this purpose, suitable solutions must be found for individual parts, they must be feasible, meet the technical re-

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quirements and not be too costly. Through this dialogue, the result should be an economically feasible concept that more than meets the necessary construction and environmental requirements. This procedure is controversially discussed, as it can inhibit or delay the construction of biogas plants. However, it must be noted that a careful examination should be carried out in order to protect the environment. Especially since faulty concepts and constructions can endanger the groundwater, the climate and the environment through the uncontrolled emission of methane, hydrogen sulphide and fermentation residues.

Biogas plant planners are also in contact with research institutions. The topics are as diverse as those between research & development and biogas plant operators.

- New technical components
- Measurement technology
- Concepts for biogas plant operation
- Feed-in models
- Additives for the microbiome

The above-mentioned and many other research topics can be developed in cooperation with research institutions. This is often done within the framework of research projects that promote innovation at biogas plants, create jobs and generally promote the development towards renewable energy sources. Therefore, this relationship between the parties involved is extremely important to drive development and make the resulting products competitive in the market.

In addition, the biogas plant planners are in contact with farmers and regional waste management companies in order to acquire more substrate if necessary and to market the resulting bio-fertiliser if the biogas plant operator does not use it himself. The feed-in tariffs are negotiated with the network operators for electricity, gas and heat in connection with the subsidy.

9.3 Authorities

The authorities have the function to check the construction of the biogas plant for compliance with the applicable environmental and building laws. For this purpose, they issue the necessary building and environmental permits after a successful inspection. For this purpose, the authorities receive specifications and guidelines from the European Union and the national governments, which must be complied with for the construction, operation and expansion of biogas plants.

In Germany, these include:

- Renewable Energy Sources Act (EEG)
- German Build Code (BauGB) with the regional building codes
- Federal Emission Control Act (BImSchG)
- 4th Federal Emission Control Ordinance (BImSchV) → List of all plants requiring approval) - decides whether a plant has to be approved according to BImSchG or building law

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- 12th BImSchV with regard to the Major Accidents Ordinance (for plants with more than 10 t of biogas)
- 44th BImSchV for CHP plants with a rated thermal input of more than 1 MW
- Technical Instructions on Air Quality Control 2021 (TA Luft)
- Technical Instructions on Noise (TA Lärm)
- Technical rules of plant safety (TRAS 120) for BImSchG plants
- Safety rules for agricultural biogas plants T4
- Ordinance on systems for handling water-polluting substances (AwSV)
- Water management law (WHG)
- Federal Nature Conservation Act (BNatSchG)
- Environmental Impact Assessment Act (UVPG)
- Veterinary approval according to Article 24 VO(EU) 1069

All these directives and regulations must be discussed in advance with the biogas plant planners and biogas plant operators, and suitable concepts must be found. The end result of this process is a plant that meets these requirements and at the same time jeopardises the economic efficiency of the entire process. The laws and regulations presented refer to Germany and are therefore noted as German federal laws. However, for each of the points: Nature protection, Water protection, Emission protection, Environmental protection, Technical instructions on noise, Air control, Substances hazardous to water and Safety of biogas plants, there are legal texts that were developed by the EU and must also be applied in all other EU countries.

The authorities act as a control instance between the European Union, the national government and the individual stakeholders. On the one hand, they adapt and implement the laws and regulations adopted by the European Union and the respective national government. On the other hand, they ensure compliance with these laws and regulations by biogas plant operators, biogas plant designers, farmers and analytical laboratories.

9.4 Research & Development

Research & Development has the task of dealing with various problems that arise at any point in the process. These can concern, for example, the microbiome of the biogas plant, chemical and microbial analysis, plant construction, various plant components, the general mode of operation, further utilisation concepts, etc.

These problems are usually dealt with in the form of research projects. With these, cooperations between research institutions, small companies and larger companies can be created, not only to improve the process, but also to create an industry network. In this network, problems can be addressed even more quickly. Research & Development is largely funded and supported by the national government and the European Union. As a result, these political institutions contribute to the research priorities and thus give direction to how science and industry should develop.

The first important link is between research & development and between biogas plant operators. Either the biogas plant operators approach Research & Development with concrete problems or samples from the biogas plant. Or Research & Development approaches the

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biogas plant operators to obtain samples from the biogas plant, information on the operation or opinions of the biogas plant operators. This connection is very important to promote further development and to initiate potential projects.

Research & Development is also in contact with the biogas plant planners. They provide each other with knowledge, ideas and networks. Both stakeholders are close to the chemical, microbial and technical processes taking place. Due to this convergence of knowledge, research projects often arise in cooperation with other stakeholders. The laboratories for chemical and microbial analysis are also among the other actors. They can provide the research institutes with samples, sample handling procedures and analytical results. In turn, they check whether the requirements are sufficient and how valid the results are.

As already mentioned, Research & Development is strongly linked to political actors such as National Governments and the European Union. These decide the budgets and how much money is spent on research programmes. In addition, the relevant committees decide which research programmes are to be funded. In this way, the political stakeholders set the direction of research and industry. In return, the research institutes regenerate valuable data and results on the corresponding research priorities. These result in recommendations for action, improvement strategies and concrete industrial products. This makes it possible to implement current efforts of the national government and the European Union. These include, for example, the Green Deal, the Waste Framework Directive, the Paris Climate Agreement, etc.

9.5 Laboratories

The laboratories carry out chemical and microbial analyses for biogas plant operators, farmers and, in some cases, Research & Development. They receive samples from the aforementioned actors and check compliance with prescribed limit values for, for example, heavy metals, nitrates, phosphates, certain bacteria and fungi. The laboratories are therefore important actors for carrying out quality control and ensuring a healthy bio-economy.

The limit values and analysis procedures are constantly reviewed, revised and adapted in cooperation with research institutions and industry. The National Governments or the European Union then establish the limit values that have been developed as mandatory requirements in environmental law. The analytical procedures are published in the form of standardised protocols and norms to ensure the validation of the data.

9.5 Farmers

The farmers are either the biogas plant operators themselves or the farmers cooperate with the biogas plant operators. In the first case, the biogas plants are operated with the farmers' own substrate and the resulting bio-fertiliser is then brought to the field and used by the farmer himself. In the other case, the farmer supplies the biogas plant operator with substrates such as animal excrement, plant residues and surplus feed. In return, the farmer receives valuable bio-fertiliser, which has to be tested by laboratories for various parameters. These are also in contact to ensure a healthy bio-economy.

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Farmers are also in contact with the National Government and the European Union. These have requirements for the use of fertilisers, pesticides, the cultivation of crops and the keeping of livestock. In addition, the construction of a biogas plant and the use of, for example, manure as a substrate is strongly encouraged in order to minimise emissions of carbon dioxide and methane. In this way, the political actors try to build up an agriculture that does not endanger the environment and protects biodiversity. At the same time, it wants to increase the appreciation of farmers and stabilise remuneration through additional income from biogas plants.

9.6 Energy grid owners & energy providers

The owners of the electricity, heat and gas grids first provide the trading platform to bring the resources generated by the biogas plant operators to the energy suppliers and consumers. Generally, energy suppliers market the energy resources of heat, gas and electricity to consumers. Consumers include the general public, industry, public institutions, etc.The grid owners provide the infrastructure to distribute the produced resources and pay the biogas plant operators the negotiated feed-in tariff. This is supported by government subsidy schemes from the National Governments and/or the European Union, which encourages the use of renewable energy sources. The use of heat is limited, because high losses occur during transport. Therefore, heat should be used locally. For the use of gas and electricity, there are no restrictions due to the well-developed network, but any transport involves losses, which is why local use is always more efficient but not always more effective. Therefore, a centralised solution makes sense for urban areas and a decentralised solution is desirable for rural areas.

9.7 General public

The general public is rather a passive part in this stakeholder analysis. However, the general public is very important, they are the consumers of renewable gas, electricity and heat. They need to express a demand for renewable energy sources to the government and the energy providers. They potentially have to pay a little more initially for the use of renewable energy sources, but they have to know what it is good for. Therefore, it is even more important that the general public shows a high level of acceptance and preference for all renewable energy sources.

The general public requires gas, electricity and heat for heating, cooking, cooling, transportation, lighting, etc. This is provided by the energy & fuel providers. For this, contracts and tariffs are negotiated, how much is paid for each energy resource.

The National Governments and the European Parliament are democratically elected by the people of Europe. In a way, the general public gives their wishes, future visions and ideas to the government to make Europe a better place. With this responsibility, governments make decisions to guide the future in this direction. The people should be given freedom, but also security and support.

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9.8 National governments

The National Government of each EU country is democratically elected by the general population with the corresponding program. This government has primarily the task of implementing the promised goals and the responsibility to comply with the coalition agreement. Besides this task, international and European directives and regulations must be implemented. In particular, the regulations issued by the European Union must be implemented directly and the directives must be implemented indirectly, otherwise penalties will have to be paid to the European Union.

The role of the national government is very individual and depends on the country. Countries adopt different funding methods, research priorities, target agreements and strategies for the expansion of biogas and bio-methane plants. Therefore the biogas landscape in Europe is so diverse. Countries like Germany have been using biogas for decades, which is why there are more than 10,000 biogas plants in this country. Other countries, such as France, are only now stepping up the expansion of bio-methane plants. For the further expansion of biogas plants, a Europe-wide strategy that addresses specific biogas would make sense.

9.9 National governments

The European Union is composed of the following institutions: European Council, Council of the European Union, European Parliament, European Commission, European Central Bank, European Court of Justice. The European Parliament is democratically elected by the people of Europe and thus exercises democratic control over all the other institutions. Together with the Council of the European Union, it is also responsible for the legislative process. The European Council sets impulses and directives. The European Commission functions as the executive or executive governmental power and is composed of one commissioner per state. The Court of Justice of the European Union acts as the judiciary.

In summary, the European Union is democratically elected and reflects the wishes, ideas and perceptions of the European people. At the same time, the European Union has the responsibility to realise these ideas on a realistic scale. The instruments of the European Union are directives, regulations, recommendations, decisions and opinions. These legislative texts, which apply to all actors in the Member States and to the Member States themselves, set the future direction of the European Union. The European Union has an influence on all the actors mentioned here, as they have to abide by the rules of the European Union. In particular, the influence of the European Union on the future stakeholders such as renewable energy producers (biogas plants), farmers and research institutions is enormous. The European Union decides through its budget what is subsidised and how much, which has a direct influence on economic efficiency and economic development. In return, the European Union achieves the set targets for e.g. the emission of greenhouse gases, the development of renewable energy sources, etc. In the best case, this relation between target setting and promotion is a win-win strategy.

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10. Prospects

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10.1 Potential improvement

10.1.1 Potential technical improvements

10.1.2 Potential biological improvement

10.1.3 Proposal for legal changes

10.2 Holistic view on the biogas market

10.3 Potential of the biogas sector

10.4 Recommendations for specific stakeholders willing to produce biogas

10.5 Other European projects that may benefit or be benefited by Micro4Biogas?

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