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: this milestone report must be considered a **draft** until the EC approval*

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

MICRO4BIOGAS:

A new international research and innovation project called MICRO4BIOGAS has just kicked off, aiming to develop **natural and synthetic microbial communities** for the production of optimised biogas. The project has been funded with €5,7M under the EU H2020 programme for a duration of four years, and it comprises **14 institutions from 6 countries**, including universities, companies and the local government of one Spanish town where a **cutting-edge biogas plant** will be built.

As **climate change** demands a fast transition towards sustainable power, the project aims to supplement existing renewable energy sources, such as wind and solar, with one that can be stored to produce heating or **electricity on demand**.

Biogas is mostly methane, with some carbon dioxide and traces of other gases, and it is produced by the decomposition of organic matter in the absence of oxygen (anaerobic digestion). Like biomass, biogas is burnt to produce energy **without adding fossil fuels to the carbon cycle**, and therefore it is a sustainable energy source.

Biologist **Manuel Porcar** is the coordinator of the project and group leader at the Institute for Integrative Systems Biology, I^2SysBio, in Spain (University of Valencia—Spanish National Research Council). He says: "Biogas is well beyond a promising technology: it is already a reality in many European countries. However, other countries still need to develop integrated solutions for their waste management and bioenergetic sectors."

Biogas production is carried out by bacteria that decompose organic matter, which can come from food waste, agricultural waste or livestock manure. Although the stages of this biodigestion are broadly known, the complete **process is considered a "black box"** due to the **complexity of the interactions** between biochemical reactions involved.

"The gaps in the knowledge of the microbial bases of the methanogenic process certainly mean that there is much room for further optimisation of the biogas sector in Europe," says Porcar. His team of microbiologists and biotechnologists from the MICRO4BIOGAS consortium will study these reactions in detail to design microbial communities—enhanced using Synthetic Biology techniques—that are optimised for very **efficient fermentation** of organic matter.

To ensure the laboratory process works at **industrial scale**, a **pilot biogas plant** will be built in Aras de los Olmos with public funding from the Spanish Ministry for Ecological Transition and from the regional government, applying the new scientific discoveries. This small town in the region of Valencia, to the East of Spain, has a population of 380 people, and is the first Spanish municipality to rely 100% on local renewable energy sources for power.

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Table of contents: Based on the headings below, the Micro4Biogas Roadmap will give a comprehensive insight into existing biogas technologies and provide more detailed information on microbial optimization strategies. In this context, the buzzword microbiome should be emphasised. The core topic of Micro4Biogas is the manipulation of anaerobic microbiomes using bioaugmentation and the adjustment of selection pressures, which could facilitate bioaugmentation. The optimisation of anaerobic microbiomes will not only be considered from a scientific and technical point of view but will also include an extensive policy analysis as well as economic and ecological aspects.

1. **State of the art**
2. **Technological description**
3. **Economic and ecological aspects**
4. **Manipulability of anaerobic microbiomes**
5. **Challenges for an AD-integrated biorefinery**
6. **Policy analysis and need for action**
7. **Prospects**

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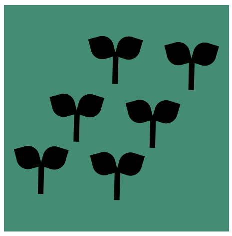
Industrial fields that are related with biogas production

Substructure of the Micro4Biogas roadmap: The AD technology is linked to wastewater treatment, waste disposal, agriculture, green waste, livestock, and the future market of new bioproducts. Throughout the study, important contributions to the respective technology fields will be **highlighted by the symbols** shown below.

Wastewater treatment: Wastewater treatment is usually divided into aerobic pre-treatment (sludge activation) and anaerobic treatment of excess sludge. Due to the aerobic pre-treatment, the energy content of the excess sludge is very low compared to other substrates for typical co-fermenters. The underlying microbiome is adapted to low concentrations of chemical oxygen demand and organic acids. Mono-fermentation of activated sludge is, like the *C. elegans* worm in medicine, a good model system for strengthening anaerobic microbiomes. When applying additional high energy substrates, the transition from a low load microbiome to a high-performance microbiome can be observed. In this relation, the question of whether digestion towers could play a role in the co-fermentation of organic residues in the future is also interesting. It should be noted that the previous statement applies above all to municipal sewage treatment plants. Industrial wastewater can be significantly more polluted. Therefore, special sludge bed reactors are often used here. This goes hand in hand with higher volume loads and shorter retention times of the substrate, which in turn affects the microbiome as well.

Waste disposal: Breaking down waste from industrial manufacturing processes and household waste is challenging for the underlying microbiome. Often there are hazardous substances that pose a risk to the environment or to the microorganisms involved in the process. This includes substances such as pesticides, petroleum-based residues and polyaromatic compounds. Aromatic compounds are difficult to degrade under anaerobic conditions and syntrophic reaction communities play a key role here. In waste treatment plants, strengthening the syntrophic reaction community through bioaugmentation could be of interest.



Agriculture: In residues from agriculture, carbohydrate-rich nutrient fractions with a high carbon content are usually used as feed for the microbiome. Examples are maize silage, grass silage or grain residues. The biggest obstacle here is the high content of lignocellulose, which requires more effort to be metabolised. Some articles are already known from the current specialist literature in which the ability to break down lignocellulose is increased by enzymatic pulping, chemical and physical pre-treatments, but also by bioaugmentation.

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Green Waste: Large amounts of green waste accumulate in municipalities, villages, and cities, which often must be disposed of for a fee. These are potential substrates for biogas plants. However, compared to residues from agriculture, the content of impurities such as stones or wood branches is high, and the energy content is extremely low compared to e.g. maize silage. New treatment methods are required here, including innovative concepts for the pre-treatment of biomass. The separation of liquid and solid components could, for example, lead to the liquid components being easily fermented and the solid components being used as fuel. The manipulation of the microbiomes involved could also help to make a larger proportion of the solid fraction available for microbial methanation.

Livestock: In the treatment of animal waste, ammonia / ammonium is a problematic substance. This is particularly an issue in poultry manure. Since poultry do not urinate, all the nitrogen is found in the poultry manure. Under anaerobic conditions, ammonia / ammonium is usually not degradable (anammox bacteria are an exception). Therefore, the mono-fermentation of poultry manure in particular leads to high ammonia / ammonium concentrations, which have an inhibiting effect on the fermentation progress. Microorganisms specially adapted to high nitrogen concentrations could provide a remedy, and based on current publications, microbial syntrophic communities are of particular importance here. With regard to microbiome manipulation, it is also important to deal with pathogens that can be enriched by animal waste.

New bioproducts: Even if it has not yet established itself industrially, there are some publications that highlight the importance of biogas plants to produce apart from methane value-added materials. A combination of the dark fermentation producing hydrogen and organic acids with a methane stage is particularly interesting. Residues from dark fermentation could be converted in methanogenic biogas plants. The acidic hydrolysate produced in the dark fermentation would have a considerable influence on methanogenic microbiomes. Even if this process is not a key topic of Micro4Biogas, at least the influence of corresponding hydrolysates on methanogenic microbiomes should be discussed in the context of the present roadmap.
Another aspect of innovation in the area of “new organic products” will be the utilization of CO_2. Despite the pollution, biogas is a concentrated point source of CO_2. Coupling anaerobic digestion to industries that require highly concentrated CO_2 could even make anaerobic digestion carbon-negative (carbon capture and storage - CCU). In general, the exploitation of industrial synergies between biogas and other industries is essential to establish a circular economy. Biogas can help to reduce emissions from a wide variety of industries, digestate can be used as building materials or fertilizer / soil-improvers and converting biogas into electricity provides heat that can be fed into the heating network. Industrial synergies are not the focus of the Micro4Biogas project but should find approval in the roadmap to convey a holistic picture of the biogas industry.

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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 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

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will be deeply characterized from several points of view: (i) innovations, technological advances and in silico analysis associated to this field; (ii) public policies and public networks involving local governments; and (iii) 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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You can also follow us on social media:

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