



Prodigio

Developing early warning systems for improved microalgae production and anaerobic digestion

PRODIGIO enhances the efficiency of solar energy conversion into biogas, paving the way for a sustainable microalgae-based biogas production industry.

Table of Contents

UNLOCKING SUSTAINABLE BIOGAS: PRODIGIO'S VISION FOR EARLY FAILURE DETECTION.....	4
ACHIEVEMENTS AND INNOVATIONS FROM PRODIGIO.....	6
1) ENHANCING MICROALGAE CULTIVATION THROUGH PATHOGEN CONTROL AND ROBUST TECHNOLOGY DEVELOPMENT FOR SUSTAINABLE BIOECONOMY.....	6
2) SUCCESSFUL ADAPTATION AND RECOVERY STRATEGIES FOR ANAEROBIC DIGESTION UNDER INDUSTRIAL PERTURBATIONS.....	8
3) LEVERAGING MICROBIAL INSIGHTS AND PREDICTIVE MODELLING TO ENHANCE LARGE-SCALE MICROALGAE CULTIVATION.....	10
4) IDENTIFICATION OF MECHANISMS AND EARLY WARNING SIGNALS FOR BIOGAS DECLINE IN ANAEROBIC REACTORS.....	12
5) COMPREHENSIVE EVALUATION OF ENVIRONMENTAL, ECONOMIC, AND SOCIAL IMPACTS OF MICROALGAL BIOGAS PRODUCTION.....	14
COLLABORATIVE INNOVATIONS: MEET THE PROJECT PARTNERS.....	16
KEY RESULTS OVERVIEW.....	18
PRODIGIO CONTACT INFO.....	19

Unlocking sustainable biogas: PRODIGIO's vision for early failure detection

OBJECTIVE

PRODIGIO is developing a system failure prediction technology to enhance the performance of microalgal biomass production and anaerobic digestion systems, aiming to improve techno-economic, environmental, and social sustainability for more sustainable biogas production from microalgae. PRODIGIO will:

- ▶ Simulate the failure of microalgae production and biomass conversion processes in lab-scale bioreactor systems.
- ▶ Develop an innovative framework for identifying early warning signals based on the analysis of causal interaction networks.
- ▶ Identify early warning signals, define threshold values, and calculate warning times.
- ▶ Evaluate the potential of early warnings to improve the economic, environmental, and social sustainability of the microalgae-to-biogas production chain.

MICROALGAE: A SOURCE OF BIOMASS FOR THE FUTURE

Microalgae are some of nature's finest examples of solar energy conversion systems, transforming carbon dioxide into complex organic molecules. Due to their outstanding photosynthetic yields, microalgae are a truly sustainable source of nutritional and bioenergy feedstock.

ANAEROBIC DIGESTION: A SOURCE OF RENEWABLE BIOGAS

Anaerobic digestion is a natural biomass degradation process carried out by microorganisms, which very efficiently transform biomass into methane-rich biogas under anaerobic conditions.

EARLY WARNING TECHNOLOGIES

Progress in bio-analytical chemistry has resulted in a vast array of chemical probes and biosensors, enabling process engineers to monitor a wide variety of parameters. However, advancing system failure prediction technologies requires identifying the most effective process parameters to guarantee failure prediction as far in advance as possible.

CONCEPT

Process failure is critical in nonlinear dynamical systems, such as bioreactors, where the functioning of complex microbial communities controls their performance. These systems undergo state transitions, shifting from one stable state to another at a critical threshold, also known as the tipping point.

Because critical state transitions alter the efficiency of microbial communities in providing services, anticipating system failure is crucial for the timely implementation of preventive measures that ensure process stability and technology profitability (Figure 1).

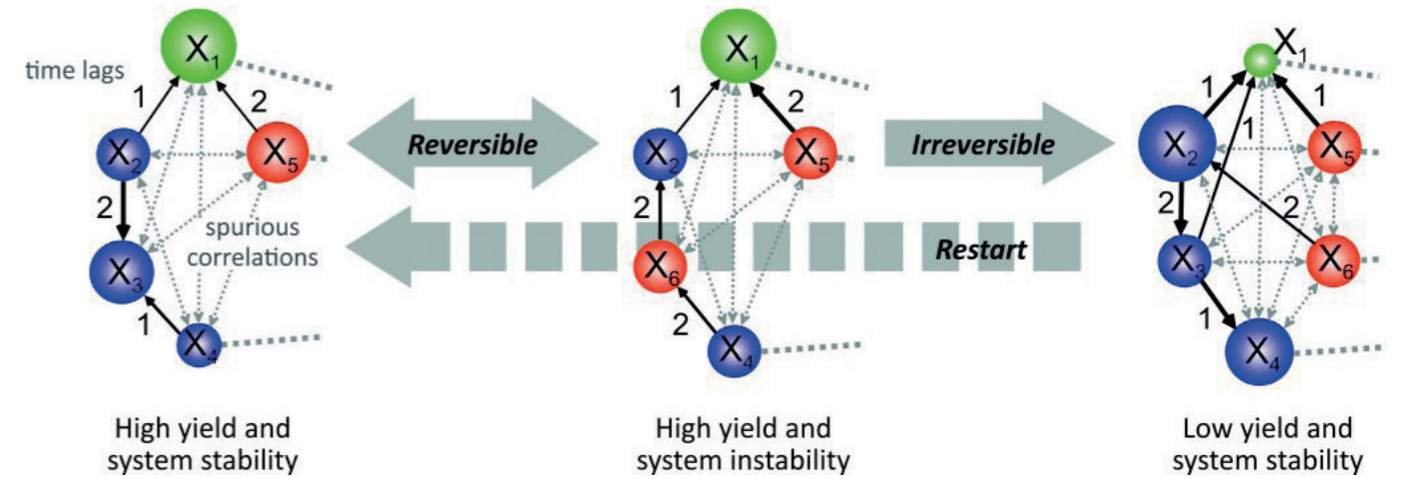


Figure 1. Theoretical scheme showing a complex, nonlinear dynamical system transitioning from the normal state to the failure state. Colours denote different components of the system, such as the end-product (green), keystone microorganisms (blue), and environmental conditions (red), interacting among them (arrows). PRODIGIO aims to identify early warning signals that allow us to anticipate as far in advance as possible when the system is going to crash.

By combining 'big data' acquisition from perturbation experiments in bioreactor systems, omics and chemical fingerprinting technologies, state-of-the-art bioinformatic tools, and novel methods for the analysis of causal interaction networks, PRODIGIO will decode triggers, identify early warnings, define threshold values, and calculate warning times for critical state transitions in bioreactor systems.

INNOVATIVE POTENTIAL

PRODIGIO innovates by:

- ▶ developing an innovative methodology for the identification of early-warning signals based on the analysis of causal interaction networks, a versatile methodology applicable to any other artificial (bioreactor) system driven by microbial communities, and
- ▶ generating a base of knowledge for the development of scalable, reliable, and affordable system failure prediction technologies that, integrated into comprehensive monitoring and control systems, help improve the performance of microalgae biomass production and anaerobic digestion systems.

IMPACTS

Expanded the genomic database of pathogens infesting microalgal culture systems and of anaerobes residing in anaerobic reactors to facilitate biotechnological advances.

Gained a further understanding of the genes, gene expression patterns, and stress response patterns of industrially relevant microbial guilds involved in microalgae biomass production, microalgal fermentation, and bio-methanisation.

Achievements and innovations from PRODIGIO

1 ENHANCING MICROALGAE CULTIVATION THROUGH PATHOGEN CONTROL AND ROBUST TECHNOLOGY DEVELOPMENT FOR SUSTAINABLE BIOECONOMY

1. Initial Challenges

Microalgae offer a promising sustainable alternative to fossil fuels and a potential solution for global food security. Their exceptional ability to convert sunlight and carbon dioxide into valuable organic compounds, such as biofuels, biochemicals, bioplastics, and high-value nutritional supplements, positions them at the forefront of green innovation. However, despite the immense potential recognized over the past two decades, large-scale microalgae production faces significant challenges that hinder the commercial expansion of microalgae-derived products. A major obstacle to large-scale production is the vulnerability of microalgae cultures to contamination by viruses, bacteria, parasites, and grazers.

To unlock the full potential of microalgae and foster the growth of related industries, the development of more robust and predictable technologies is crucial. Advancements in this area would not only address the existing challenges but also pave the way for the widespread adoption of microalgae-based solutions in various sectors, contributing to a greener and more sustainable future.

2. Solutions Implemented

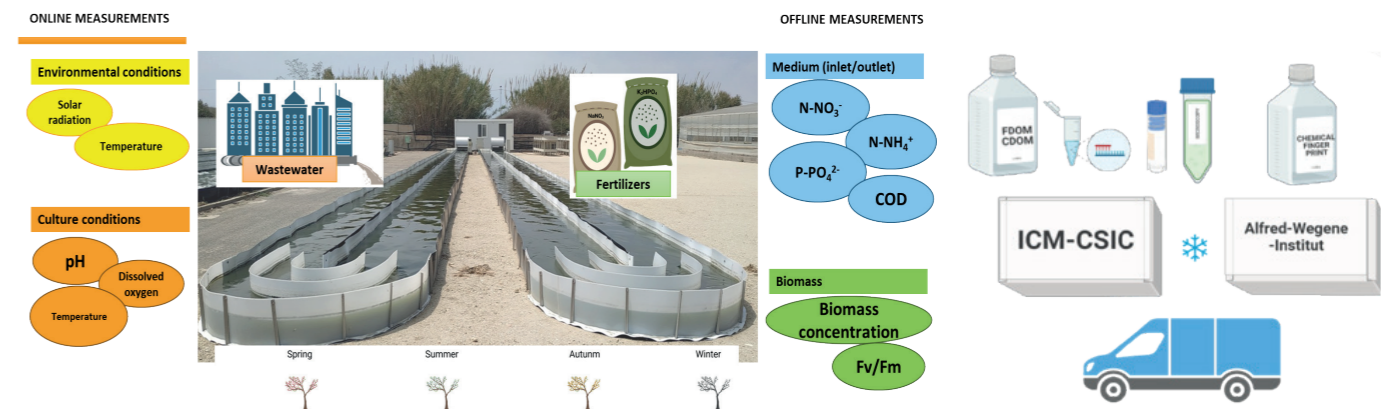
In the PRODIGIO project, the continuous operation of microalgae raceway reactors under real outdoor conditions was investigated. Experiments utilised both clean water and fertilisers for food/feed-grade biomass production, as well as effluents for lower-grade biomass suitable for energy purposes, while also contributing to nutrient recovery and greenhouse gas mitigation. The research confirmed that, similar to crops like wheat and maize, most failures in microalgae cultivation are linked to pathogens such as fungi, pathogenic bacteria, and viruses. This suggests that microalgae production shares commonalities with traditional agriculture, opening possibilities for developing analogous control strategies.

Identifying the most significant pathogens damaging microalgae cultures opens the door for the development of control strategies that minimise system failures. For example, new monitoring methods are being developed for the early detection of pests and diseases. Additionally, the identification of novel environmentally-friendly pesticides derived from the activity of the algae themselves or their associated microbiomes is another promising avenue for improving microalgae cultivation. Companies and research centres are actively combining their efforts in this respect.

3. Conclusions and Results

The completion of planned activities confirms the relevance of improving our understanding of the biological systems involved in microalgae-related industrial processes. Environmental variables such as solar radiation and temperature determine the performance of microalgae cells but also the emergence of algae-pathogens such as fungi, bacteria and viruses. Fungi and pathogenic bacteria have been identified as major culprits in the deterioration of outdoor microalgae cultures. Understanding the interactions between the target microalgae and their microbiomes (detailed on the page 10) is crucial for maintaining optimal culture health and maximising biomass production. Likewise, alternative strategies such as the adjustment of pH or dissolved oxygen concentrations may also contribute to ensuring performance stability and facilitating the widespread deployment of large-scale microalgae production systems.

The advances developed as part of the PRODIGIO project will help to improve the reliability of microalgae-related industrial processes. Microalgae are recognized as a safe and sustainable source of food and feed, and they also hold significant potential for enhancing the EU Bioeconomy through the production of bioplastics, biostimulants/biopesticides, and biofuels, among other valuable products. Expanding the reliability of microalgae industrial processes will undoubtedly contribute to the overall sustainability of the EU Bioeconomy.



Scheme of the experimental settings using outdoor raceway reactors and analytical measurements. Samples for DNA sequencing and high resolution mass spectrometry were shipped to partner laboratories at the ICM-CSIC in Barcelona (Spain) and the AWI in Bremerhaven (Germany), respectively.

2 SUCCESSFUL ADAPTATION AND RECOVERY STRATEGIES FOR ANAEROBIC DIGESTION UNDER INDUSTRIAL PERTURBATIONS

1. Initial Challenges

WP2 aims at exploring the anaerobic digestion (AD) failure against several perturbations commonly found at industrial scale. The objective is to determine the indicators that could lead to unravelling early indicators of biogas production decline.

The scaling-up of AD presents a significant challenge due to the systematic efficiency losses related to process instabilities. WP2 was designed to establish the basis required for the development of a failure prediction system that increases the performance of AD technology. The ultimate goal is thus to provide advances towards a more favourable techno-economic, environmental and social performance of biotechnological-based processes to achieve more sustainable biogas production from AD of microalgae biomass.

2. Solutions Implemented

To evaluate the AD behaviour against perturbations and identify the indicators that could evidence a biogas production decline at an early stage, 6 anaerobic reactors (ARs) were operated in parallel (Figure 1) using enzymatically pre-treated microalgae biomass (*Scenedesmus* sp.) as feedstock.



Figure 1. Photo of the experimental setup.

ARs were inoculated with sludge collected from a conventional anaerobic digester located in a wastewater treatment plant (El Soto, Móstoles, Madrid-Spain). ARs were equipped with online sensors to monitor pH, temperature and oxidation-reduction potential. Likewise, biogas production was daily measured using a flow metre. Initially, ARs were conducted under identical operational conditions until all the chemical output showed steady-state operation. At that time, operational conditions were modified to apply several perturbations in parallel, while keeping control ARs (non-perturbed reactors) also running. The studied perturbations are listed in Table 1.

Table 1. Studied perturbations in ARs.

Group	# Experiment	Type of perturbation	Shock period (d)	Experimental time (d)	Description
OLR	1	Sudden OLR increase	10	195	Sudden OLR increase from 1.5 to 3 g COD/Ld. After 10 days of shock, the conventional OLR was suddenly imposed again until recovering the process.
		Sudden OLR increase	10		Once the process was recovered, the OLR was suddenly increased again from 1.5 to 7 g COD/Ld. After 10 days of shock, the conventional OLR was suddenly implemented until recovering the process.
	2	Sudden OLR decrease	10	195	Sudden OLR decrease from 1.5 to 0,75 g COD/Ld. After 10 days of shock, the conventional OLR was suddenly implemented until recovering the process.
Sudden OLR decrease		10	Once the process was recovered, the OLR was suddenly decreased again from 1.5 to 0 g COD/Ld. After 10 days of shock, the conventional OLR was suddenly implemented until recovering the process.		
Microalgae composition	4	Stepwise microalgae composition change	45	157	Stepwise OLR increase from 1.5 to 7 g COD/Ld for 45 days. Thereafter, the OLR was suddenly decreased to conventional level (1.5 g COD/L) until the process exhibited a complete recovery.
					Stepwise protein content decrease in the biomass (w/w) from 52 % (14 % carbohydrates) to 28 % (62 % carbohydrates) for 45 days. Thereafter, the microalgae biomass used as feedstock was again the one rich in proteins (52 %).
Toxics / inhibitors	5	Presence of pesticide	15	88	30 µM of Rotenone (Van Ginkel et al., 2020) was dosified for 15 days along with the microalgae biomass. Thereafter, pesticide-free microalgae biomass was used as feedstock again.
	6	Presence of antibiotic	10	68	1.5 g/L of tetracycline (de Godos et al., 2012) was dosified for 10 days along with the microalgae biomass. Thereafter, antibiotic-free microalgae biomass was used as feedstock again.
	7	Salinity intrusion	15	156	Microalgae biomass concentration was adjusted using saline water from the Mediterranean sea to simulate a salinity intrusion for 15 days. Thereafter, conventional microalgae biomass was used as feedstock again.



These experiments allow us to accomplish the tasks proposed in WP2 and provide data to develop a prediction model to anticipate AD failure, as designed in WP4.

Dr. Cristina González-Fernández and Dr. Silvia Greses designed and performed the AD experiments at IMDEA Energy facilities (Figure 2), providing samples to analyses the microbiomes behaviour at NMBU (Norway) and determine chemical fingerprinting at AWI (Germany). Moreover, time-series data were shared with NTU (Taiwan) to develop the prediction model and Armines (France) to perform a dynamical life cycle assessment.

Figure 2. Dr. Cristina González-Fernández (left) and Dr. Silvia Greses (right)

3. Conclusions and Results

The experiments related to AD perturbation showed that the anaerobic microbiome can adapt to fluctuations in microalgae composition. However, the AD response to organic overloading is contingent on the length and severity of the perturbation, which affects different steps of the process.

Additionally, the study of chemicals impact on AD revealed that specific pesticides used to control algae grazers are harmless to the AD microbiome.

In contrast, antibiotics demonstrated a higher toxic potential than salinity when evaluating chemicals potentially present in microalgae biomass. The process inhibition should be considered in all steps of AD, including acidogenesis and acetogenesis. Ethanol has been confirmed as a reliable indicator of acidogenesis failure, indicating an AD unbalance and leading to a decline in methane production. Regarding AD recovery, antibiotics and salinity disturbances hinder the natural recovery of AD systems, needing alternative strategies to restore microbiome activity.

Antibiotics, in particular, caused significant damage to the AD metabolic network. However, a faster recovery of the AD system can be achieved by reinoculating the anaerobic digester with an adapted microbial system.

3 LEVERAGING MICROBIAL INSIGHTS AND PREDICTIVE MODELLING TO ENHANCE LARGE-SCALE MICROALGAE CULTIVATION

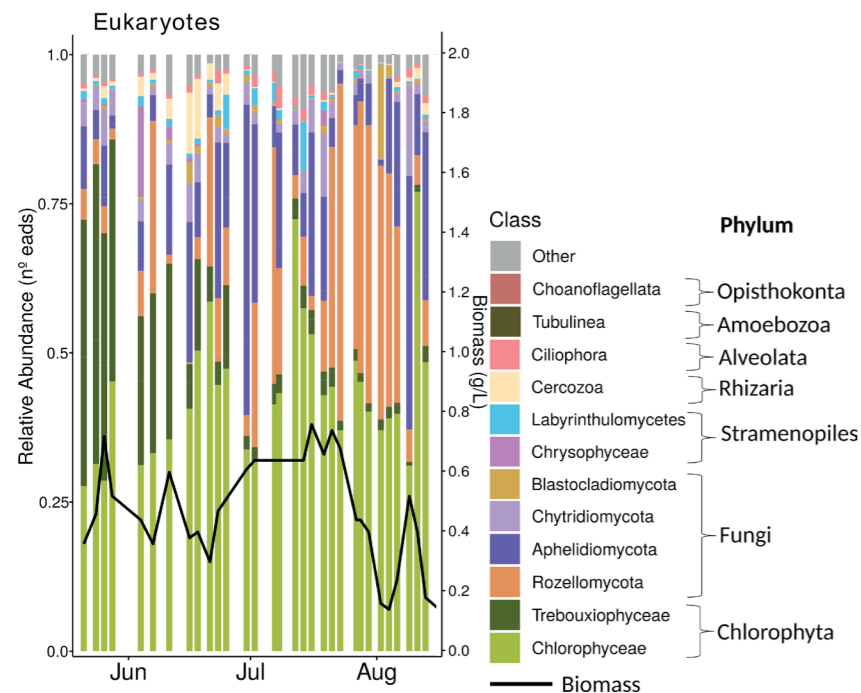
1. Initial Challenges

Advanced sequencing technologies are revolutionising our understanding of microbial ecosystems by uncovering the enormous diversity and complex interactions among microorganisms. This insight underscores the importance of managing the algae-associated microbiome – the community of microorganisms that grow and interact with the microalgae – as a strategy to anticipate system failures and enhance productivity. This microbiome isn't just a bunch of random microbes; it's like a fingerprint for the health of the microalgae ecosystem. By looking at the types of microbes present and their genetic makeup, we get a snapshot of how well the whole system is doing. But there's a catch: as we grow microalgae on a larger scale, keeping their microbiome healthy becomes trickier. The bigger the system, the more complex it gets, and all sorts of things can throw the microbes off balance. Environmental factors, pathogens or diseases can disrupt the delicate harmony between the microalgae and their microbial partners. Understanding the interactions between microalgae and their microbiome is critical to detecting early warning signals of system failure (or dysbiosis) and devising ways to keep microalgae thriving.

2. Solutions Implemented and Results

In PRODIGIO, we've been studying the microbial communities in large-scale microalgae cultivation systems over two nine-month periods, one in 2021 and another in 2022. One system was fed with wastewater, while the other received clean water and fertilisers. Metabarcoding analyses targeting the 18S and 16S rRNA genes unveiled an astonishing variety of prokaryotic and eukaryotic microorganisms in these systems. And not only that, but the types and amounts of microbes changed a lot over time.

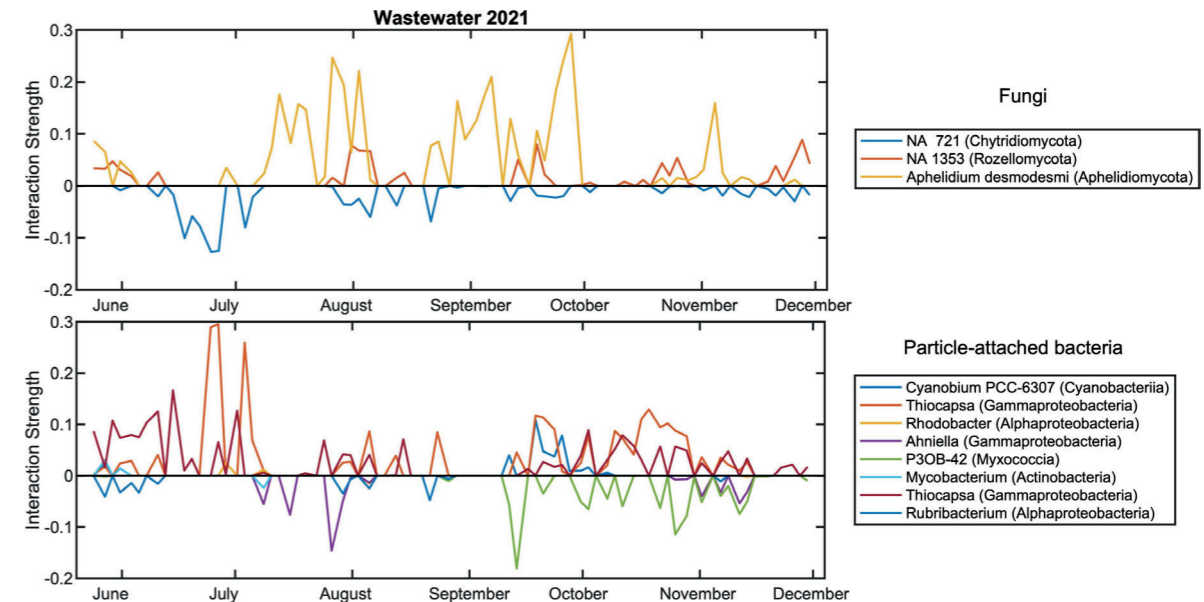
To go a little further from a simple description of microbial dynamics, in PRODIGIO, we have applied Empirical Dynamic Modeling (EDM), a data-driven approach for modelling complex and nonlinear dynamical systems, to the time series of microbial dynamics. EDM allowed us to infer cause-effect interactions between microorganisms and explore how these interactions varied over time. These time-varying interactions provide invaluable insights into the dynamical evolution of microalgae systems, enabling the forecasting of system behaviour.



Eukaryotic diversity within the microalgae production systems.

Microbiome class-level identifications of eukaryotic sequences. Results based on 18S rRNA region V4 are shown in order of increasing abundance within wastewater raceway for the beginning of the 2022 study. The ten more abundant classes are listed and the other groups are grouped into the "other" class (gray bars). Superimposed is the concentration of biomass in the system.

Simultaneously, through metagenomics and bioinformatics, we are unravelling the complex nature of these microbial interactions at the molecular level. For instance, our bioinformatic pipelines are enabling us to reconstruct the metabolic exchange between microalgae and other key microorganisms. This detailed understanding of metabolic exchanges and their effects lays the foundation for targeted genetic manipulation. For instance, gene editing tools can be employed to precisely modify genes responsible for microalgae cell wall composition and integrity, thereby enhancing their resilience against pathogen attacks.



Microbial interactions in the microalgae production systems.

Interactions of common fungi and particle-attached bacteria on *Desmodesmus armatus*; the most common *Desmodesmus* sp. in the bioreactors, inferred from EDM analyses.

3. Conclusions and impact

Understanding the complex dynamics between microalgae and their associated microbiomes is a significant challenge, but one that promises to unlock new possibilities for managing microalgae culture systems effectively. In the PRODIGIO project, we have leveraged cutting-edge mathematical modelling techniques to unravel fascinating interactions between microalgae, bacteria, and fungi.

Understanding the interactions between microalgae and associated microorganisms is vital for detecting early signs of stress or ecological imbalances (dysbiosis). The use of specific probiotics and the control of culture conditions could help preserve the algae microbiome and optimise the benefits it provides.

Finally, genomic information on microalgae and their microbiomes opens the door to developing sophisticated predictive models using artificial intelligence (AI). These models are powerful tools that allow for the simulation of the effects of genetic engineering and microbiome manipulation before their actual implementation in real-world environments. By anticipating how these alterations can influence the productivity of cultivation systems, AI will significantly accelerate the cycle of innovation in biotechnology, optimising the development of more resilient and productive systems. This represents a promising avenue for future research, leveraging the extensive genomic and biochemical databases created during the PRODIGIO project.

4 IDENTIFICATION OF MECHANISMS AND EARLY WARNING SIGNALS FOR BIOGAS DECLINE IN ANAEROBIC REACTORS

1. Initial Challenges

The overall goal of WP4 has been to decipher the underlying mechanisms for biogas decrease in anaerobic reactors (ARs) fed with microalgal substrates and identify early warning signals using an innovative method based on the analysis of causal interaction networks. To be able to reach this goal, we had to link data from multiple sources, including community structure (16S rRNA gene metabarcoding), functional gene potential (metagenomics) and -expression (metaproteomics), bioreactor parameters (pH, volatile fatty acids, methane yield), and chemical fingerprints (metabolomics). A large undertaking by multiple labs across Europe was needed to operate the reactors, extract samples, facilitate shipping between labs, and analyze all the samples.

2. Solutions Implemented

The first task of WP4 was generating tables containing all the various data in a format capable for integrative analysis. At this point we had tables of operational taxonomic units (OTUs) with abundance profiles, metagenome-assembled genomes (MAGs) with metabolic reconstructions and functional annotations as well as expression data, lists of chemical parameters of bioreactors fluctuating throughout the AR's operation time, as well as lists of chemical formulas identified from metabolomics with abundances over time in various reactors and treatments. Thousands of lines of code were written in R to unify and link the data, generating a knowledge database for further analysis. This dataset was then utilized for integrative analysis looking at specific biological processes known to occur in ARs, simulation of process modifications to predict process performance, as well as unsupervised system failure prediction technology through empirical dynamic modelling.



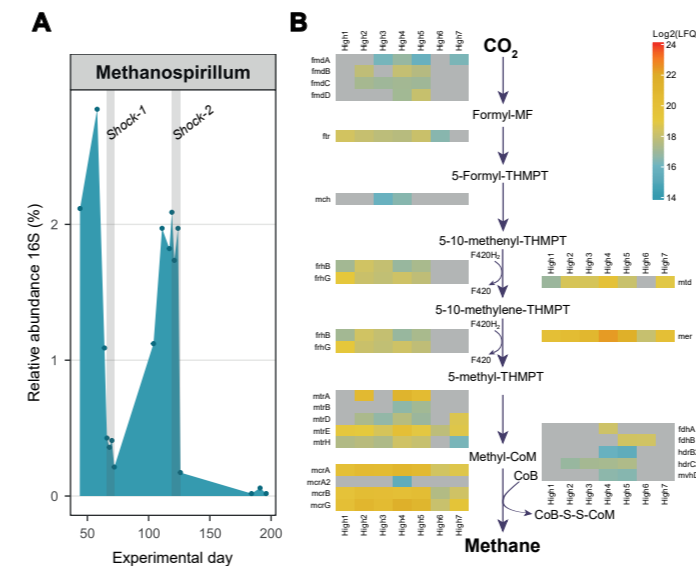
NMBU PRODIGIO-team. Top-middle: Senior researcher Live H. Hagen and post doc Juline Walter preparing samples for metaproteomics using state-of-the-art mass spectrometry. Top-right: PhD student Alessandra Ferrillo loading an Oxford Nanopore flow cell for long-read DNA sequencing. Bottom: PhD student Valerie Schiml doing bioinformatics of integrated metagenomics and metaproteomics data. Left: The NMBU team, also including Research Professor Magnus Ø. Arntzen, who is leading work-package four.

3. Conclusions and Results

Integrative analyses and modelling

The knowledge database of integrated data allowed us to identify changes in the abundance of microbial populations as response to reactor perturbations and correlate these with the functional roles of specific microbes in the reactors. We observed clear changes in microbial profiles during the shock-phases, both for organic over/under load and for high/low protein load. As the reactors went from steady state to shock to critical failure, clear signatures could be observed and correlated, such as the drop in methane yield and the decline of specific methanogens, but also changes in the abundance of non-methanogens, i.e., responsible for upstream processes, such as hydrolysis, acidogenesis and acetogenesis.

Modelling revealed that the inhibition of hydrolysis, acidogenesis and acetogenesis took place during the overloading perturbation. This conclusion was drawn because simulations based solely on the inhibition of methanogenesis, using the traditional Anaerobic Digestion Model No. 1 (ADM1, IWA), failed to reproduce the experimental data accurately. Our implementation of two new inhibition-by-product functions into the hydrolysis and acidogenesis/acetogenesis stages allowed us to simulate anaerobic digestion behaviour during the stable, perturbed, and recovery periods. Hence, the modified ADM1 successfully predicted process performance against one of the most common perturbances found at industrial scale.

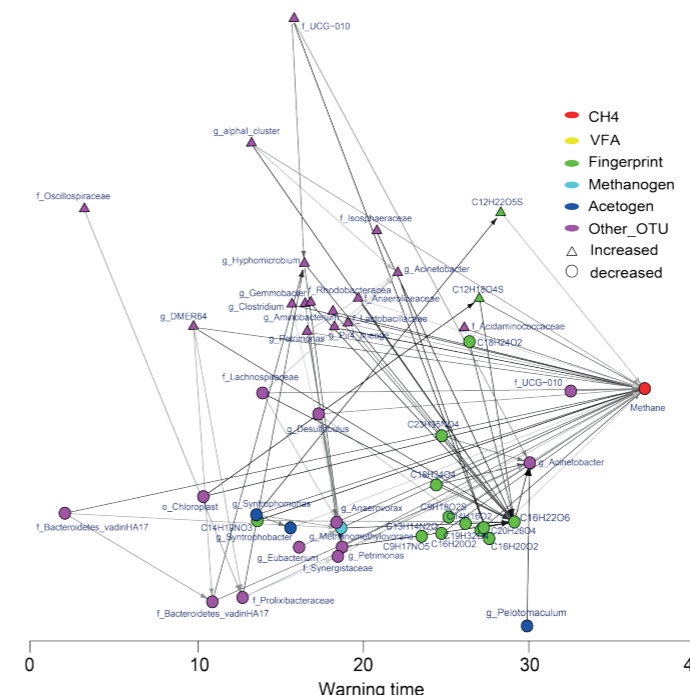


Methanospirillum is a key methanogen in the bioreactors and responsible for converting CO2 to methane.

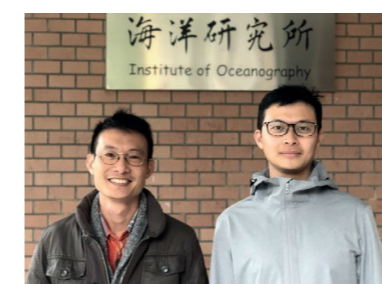
The population of *Methanospirillum* is sensitive to the high organic load, as indicated with shock 1 and 2, causing a steep decline in the population (A). Metaproteomics in combination with metagenomics allowed us to quantify all the relevant enzymes of the methanogenesis pathway for this specific genus and follow their abundance over time through reactor transitions from steady state to shock conditions (B). The steep decline observed in Shock-2 is also evident at the enzyme-level in samples High 6-7.

Identification of early warning signals

Empirical dynamic modelling (EDM) provided us with an effective early warning signal to anticipate bioreactor failure (i.e., critical transition in methane production) caused by long-lasting organic stress. Furthermore, the use of EDM to reconstruct the interaction networks between key microbes and metabolites offered a mechanistic view for the development of critical transition in methane production. Specifically, we revealed the strengthened negative impacts from volatile fatty acid on methanogens and enhanced positive feedback when approaching the tipping point, implying the loss of resilience for maintaining methane production. Through tracking the interactions that contributed to the abrupt shift in methane production near the tipping point, we unveiled a series of cascading changes among microbes and metabolites that occurred earlier than bioreactor failures. These findings suggested a promising research direction for anticipating the occurrence of critical transition based on more mechanistic-, network-based warning signals that send the warnings even earlier than the existing phenomenological early warning signals.



Domino-like cascading mechanisms drive the critical transition in methane production. We evaluated the contribution of specific nodes to the dramatic decline in methane production at the tipping point (Day 37). Methane reduction can be a consequence of either weakened positive effect or strengthened negative effect. However, for those nodes contributing to methane collapse, they could also undergo significant changes at earlier stages. Therefore, the changes in those nodes can subsequently be attributed to the effects of other nodes, and so on. As such, we determined numerous paths that drive the significant changes in the nodes involved (like dominos) and eventually lead to a critical decline in methane production (the last domino). Here, the colors of the nodes label their functional identities; the horizontal position of the nodes was arranged according to the timing in which significant changes occurred in their states. For simplicity, we only present those nodes that also underwent critical transition during the sampling period of the contribution.



NTU PRODIGIO-team. Professor Chih-hao Hsieh (Left) and assistant professor Chun-Wei Chang (Right) in front of the Institute of Oceanography, National Taiwan University.

5 COMPREHENSIVE EVALUATION OF ENVIRONMENTAL, ECONOMIC, AND SOCIAL IMPACTS OF MICROALGAL BIOGAS PRODUCTION

1. Initial Challenges

Despite the potential of microalgae as a renewable source to produce energy and the opportunities of coupling this function with other activities such as wastewater treatment, microalgae processes also entail the consumption of materials and energy. This can involve a non-negligible level of resource depletion and emissions of pollutants, as well as positive and negative socio-economic effects. Its economic viability also needs to be assessed.

WP5 of PRODIGIO project aimed to evaluate the potential environmental, economic and social consequences of energy production from microalgal biogas, using a life cycle perspective and considering the following challenges:

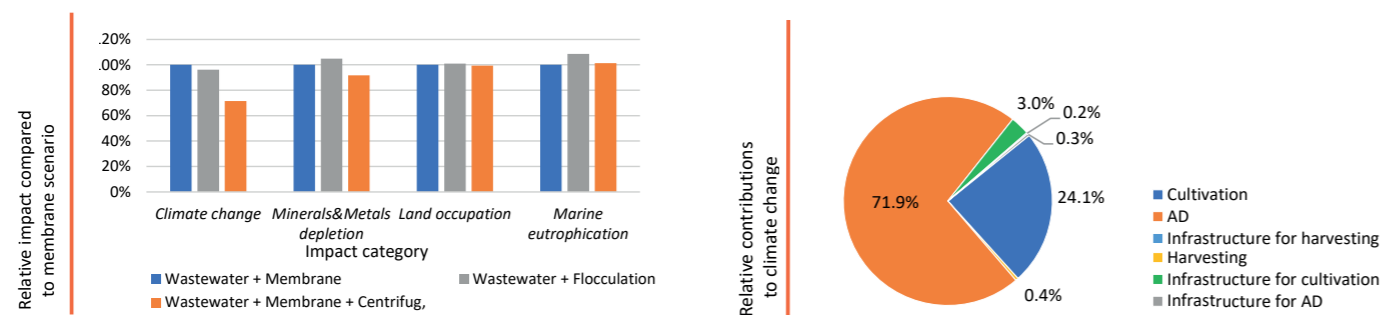
- ▶ The dynamic nature of microalgae processes (e.g. time-dependence of productivity or composition due to variable temperatures and solar irradiation) is not fully accounted for in most Life Cycle Assessments published to date.

- ▶ The social dimension of microalgae bioenergy has been scarcely assessed.

- ▶ The application of life cycle approaches is hindered by the difficult access to representative data due to the low TRL of certain technologies.

2. Solutions Implemented

The potential effects of microalgal biogas on the environmental, social and economic dimensions of sustainability were evaluated using Life Cycle Assessment (LCA), an internationally recognized method to evaluate the potential impacts of products and systems over their life cycle, from the raw materials' extraction and transformation to the use and end-of-life. Originally focused on environmental aspects, LCA has been extended to cover social and economic aspects.



In Prodigio, the assessment of the three dimensions has been conducted in parallel. For all the dimensions, data have been collected from Prodigio partners, mainly UAL and IMDEA Energy. For the environmental LCA, a parameterized approach based on a LCA model built in Python programming language has been used. For the economic assessment, collected data were completed with simulations in Aspen software, which allowed the mass and energy balances to be estimated. For the social LCA, a model was built using the software openLCA and the PSILCA database.



3. Conclusions and Results

The developed models allowed the comparison of biogas production scenarios and the identification of main contributors to potential impacts.

The effect of the integration of dynamic aspects in the modelling of the environmental LCA depends on the scenario. Significant differences between static and dynamic approaches were observed for all impact categories when two consecutive perturbations increasing the organic loading rate during the anaerobic digestion were considered. For other scenarios, differences were only found for some categories (e.g. freshwater eutrophication). Scenarios with harvesting via membrane filtration plus centrifugation were identified as having the lowest environmental impacts. Early warning systems enhanced the performance compared to scenarios facing the perturbation without them. Cultivation (mainly linked to CO₂ supply) and pretreatment (linked to enzymes production and, for some scenarios, heat) phases were identified as the main contributors to the impacts.

The Life Cycle Costing estimated in 16.8 M€ the fixed capital investment required for the design of a hypothetical commercial plant. Considering a 10-year lifetime of the facility, the process could present a viable economic performance for a minimum selling price of 100 €/MWh under the assumed operation conditions. Anaerobic digestion was the main contributor to costs, with the cultivation being responsible for less than 20% of the total production costs.

For the social dimension, workers and local communities were identified as the two stakeholder categories for which impacts should be analyzed in priority according to different perspectives and also to the number of impact categories shown in the literature. Results show that the main contribution to social risks (60–80%) is the anaerobic digestion section, to a large extent linked to manufacturing sectors within Europe such as chemicals' production, metal products manufacture and other machinery.

Overall, results from this WP may help put potential consequences of Prodigio target systems' implementation and provide hints to decision-makers for further development. Future research should enhance the application of dynamic LCA modelling, for example by considering not only the dynamics of the operation but also of the pollutants' emissions. The integration of environmental, economic and social results into a common interpretation phase should also be further developed.

Collaborative innovations

Meet the project partners:



Spanish National Research Council (CSIC)
Consejo Superior de Investigaciones Científicas

As Spain's largest public research institution and a key player in European research development, CSIC brings extensive expertise in microbiology and project management. Their participation in the PRODIGIO project involves two leading research centres: the Institute of Marine Sciences in Barcelona and the Centre of National Biotechnology in Madrid, contributing specialized knowledge in microbial ecology to advance eco-genomics in microalgae biomass production systems.



The Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research (AWI)
Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar- und Meeresforschung

As a renowned scientific institution dedicated to polar and marine research, conducting comprehensive studies in the Arctic, Antarctic, and adjacent coastal regions, AWI brings interdisciplinary expertise to the PRODIGIO project. Specifically, AWI's Department of Ecological Chemistry focuses on chemical fingerprinting of organic matter, providing valuable insights into microbial community dynamics.



Association for Research and Development of Methods and Industrial Processes (ARMINES)
Association pour la recherche et le développement des méthodes et processus industriels

ARMINES is a French research organization specializing in collaborative research between industry and academia. As a member of EARTO and the Carnot Institute, ARMINES is a key player in European projects, contributing expertise in renewable energy resources and environmental impacts. In the PRODIGIO project, ARMINES, alongside MINES ParisTech, defines methods for evaluating environmental and health impacts of energy systems based on a life cycle assessment approach.



IDConsortium is a Spanish company specializing in the integrated management, dissemination, and exploitation of R&D and innovation projects. With a focus on internationalization and collaboration, IDC plays a crucial role in project design, planning, and financial management. In the PRODIGIO project, IDC's expertise ensures efficient progress tracking and effective communication and dissemination of project outcomes.



IMDEA Energy Institute (IMDEA-E)
Instituto IMDEA Energía

IMDEA-E is a leading research foundation excelling in technology transfer to the industrial sector and the development of clean and renewable energy technologies. Their Biotechnological Processes Unit (BTPU) brings interdisciplinary expertise to PRODIGIO, driving innovation in microalgae exploitation for biochemicals and biomethane through anaerobic digestion processes.



National Taiwan University
臺灣大學

NTU, a leading university in Asia, boasts a robust marine research division committed to studying and safeguarding marine ecosystems. Their expertise in theoretical ecology and EcolInformatics contributes significantly to the PRODIGIO project, focusing on microbial interactions and functions through innovative data analysis methods.



Norwegian University of Life Sciences (NMBU)
Norges miljø- og biovitenskapelige universitet

NMBU is a leading agricultural university in Norway, renowned for its research in environmental sciences, veterinary medicine, and biotechnology. With expertise in developing renewable energy technologies and sustainable aquaculture feed ingredients, NMBU's contributions to the PRODIGIO project will focus on harnessing microbial consortia within bioreactors for efficient biofuel production and sustainable aquaculture practices.



University of Almería (UAL)
Universidad de Almería

The UAL is a renowned public research institution recognized globally for its expertise in microalgae biotechnology and photobioreactor design. In the PRODIGIO project, UAL focuses on algae biotechnology and bioprocess engineering to enhance microalgae biomass production systems through innovative reactor designs, ensuring the success of perturbation experiments to simulate biomass production process failures.



Developing early warning systems for improved microalgae production and anaerobic digestion

PRODIGIO enhances the efficiency of solar energy conversion into biogas, paving the way for a sustainable microalgae-based biogas production industry.

1. FAILURE TESTS IN PHOTOBIOREACTORS

OBJECTIVE	KEY RESULTS	CONCLUSION
Investigate failure mechanisms in photobioreactors used for microalgae cultivation.	<ul style="list-style-type: none"> Identified pathogens (fungi, bacteria) that damage microalgae cultures. Developed monitoring methods for early detection of pests and diseases. Confirmed environmental impact (solar radiation, temperature) on microalgae performance and pathogen emergence. 	Enhanced reliability of microalgae industrial processes, contributing to the EU Bioeconomy by producing bioplastics, biofuels, and biostimulants.

2. FAILURE TESTS IN ANAEROBIC REACTORS

OBJECTIVE	KEY RESULTS	CONCLUSION
Explore anaerobic digestion (AD) failure under various industrial perturbations.	<ul style="list-style-type: none"> The anaerobic microbiome can adapt to microalgae composition changes. AD response to organic overloading varies with the length and severity of the perturbation. Certain pesticides are innocuous for AD microbiomes, whereas antibiotics are more toxic. Identified EtOH as an indicator of acidogenesis failure. 	Prediction model for AD failure developed for improved biogas production performance and sustainability.

3. MODELLING THE FAILURE OF MICROALGAE PRODUCTION

OBJECTIVE	KEY RESULTS	CONCLUSION
Model interactions in large-scale microalgae cultivation systems to predict behaviour and failures.	<ul style="list-style-type: none"> Applied Empirical Dynamic Modeling (EDM) to infer cause-effect interactions between microorganisms. Identified critical interactions and early system stress / imbalance signs. 	Optimised microalgae cultivation, making systems more resilient and productive.

4. MODELLING THE FAILURE OF ANAEROBIC DIGESTION

OBJECTIVE	KEY RESULTS	CONCLUSION
Decipher mechanisms for biogas decrease in anaerobic reactors and identify early warning signals.	<ul style="list-style-type: none"> Created integrated datasets for comprehensive analysis. Developed models revealed inhibition processes during organic overloading. EDM identified early warning signals and mechanisms driving biogas production decline. 	Enhanced predictability and stability of anaerobic digestion processes, critical for maintaining methane production.

5. SUSTAINABILITY ASSESSMENT

OBJECTIVE	KEY RESULTS	CONCLUSION
Evaluate the environmental, economic, and social impacts of microalgal biogas production using Life Cycle Assessment (LCA).	<ul style="list-style-type: none"> Identified major environmental impact contributors (cultivation, pre-treatment). Developed models for comparing different biogas production scenarios. Determined economic viability and social impacts, highlighting the need for dynamic LCA models. 	Informed decision-making for sustainable practices and economic feasibility.

Contact info



www.prodigio-project.eu



@prodigioproject



Prodigio Project



@ProdigioProject



@prodigioproject



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 101007006

