PRODIGIO Newsletter

DEVELOPING EARLY-WARNING SYSTEMS FOR IMPROVED MICROALGAE PRODUCTION AND ANAEROBIC DIGESTION

2021/22

THIS PROJECT HAS RECEIVED FUNDING FROM THE EUROPEAN UNION'S HORIZON 2020 RESEARCH AND INNOVATION PROGRAMME UNDER GRANT AGREEMENT NO 101007006

What is in this newsletter?

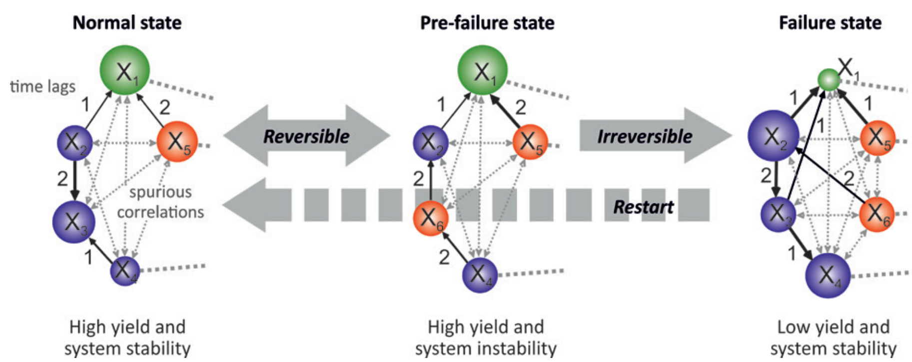
The PRODIGIO project is structured in 8 work packages. WP 1 and 2 are experimental and focus, respectively, on microalgae biomass production and anaerobic digestion processes. WP 3 and 4 use data generated by the experimental WPs and aim to model, using advanced time-series data analysis, the causes of the failure of microalgae biomass production and biomass-to-biogas conversion processes. WP 5 is designed to assess the sustainability of the microalgae biomass-to-biogas production chain explored in PRODIGIO from a techno-economic, environmental and social perspective. Finally, there are three additional WPs dedicated to the communication of results (WP6), project management (WP7) and compliance with ethical requirements (WP8).

In this first edition of the PRODIGIO newsletter, we share information on the main activities carried out in the project and the tasks in progress as of March 2022.

Check out our website and social media platforms and see what is happening in PRODIGIO project

About PRODIGIO

- PRODIGIO is a three-year Research and Innovation Action funded by the EU H2020 Framework Programme under the topic "Developing the next generation of renewable energy technologies".
- One of the main barriers to the commercialization of microalgae biomass for low-price commodities, such as food, feed or biofuels, is the low efficiency of the microalgae biomass production systems. Microalgae have enormous potential to become a truly sustainable source of biomass for the future. This is because of their high photosynthetic conversion efficiency, up to 11% (i.e. 11% of the light hitting the cells is eventually converted into biomass), as compared to 2-3% achieved by terrestrial plants. Furthermore, microalgae can grow in barren land and using non-potable water sources.
- Like terrestrial crops, microalgae biomass production systems are strongly impacted by pests and diseases, including viruses, pathogens, parasites and predators, which can spoil microalgal crops in a matter of days. Decades of agricultural research have enabled the development of early warning technologies, which, along with the development of mitigation measures (pesticides), have led to previously unthinkable crop yields. These early warning technologies are underdeveloped as long as microalgae production is concerned.
- Anaerobic digestion is the most economically-attractive process for the production of biofuel from microalgae biomass since it does not require the drying of biomass, allows the recovery of essential nutrients and it is a relatively simple procedure from an infrastructure perspective. Anaerobic digestion is a natural biomass degradation process carried out by microorganisms, which transform the organic matter into methane-rich biogas in the absence of oxygen. The performance of the anaerobic conversion process depends on the functioning of complex microbial communities. Changes in the ecological and biochemical linkages within these communities can lead to process failure, thus limiting biogas production per unit of feedstock supplied.
- In PRODIGIO, we aim to establish a base of knowledge for the development of system failure prediction technologies that increase the performance of microalgae production and anaerobic digestion systems and advance towards more favourable techno-economic, environmental and social performance to achieve more sustainable microalgae biogas. By combining perturbation experiments in bioreactor systems and cutting-edge methods for big data analysis, PRODIGIO will decode the triggers, identify early-warnings, define threshold values and calculate warning times for critical state transitions in bioreactors.



Theoretical scheme showing a complex, non-linear dynamical system transiting from the normal state to the failure state. Colors denote different components of the system, such as, the end-product (green), keystone microorganisms (blue), 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.

The results of PRODIGIO will pave the way for moving the entire microalgae to biogas production chain efficiently towards its theoretical maximum, enabling the development of a fully integrated and truly sustainable biogas production industry and contributing to strengthening the EU's leadership in renewable fuel technologies.

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Prodigio Structure and first results

Failure tests in vphotobioreactors (WP1)

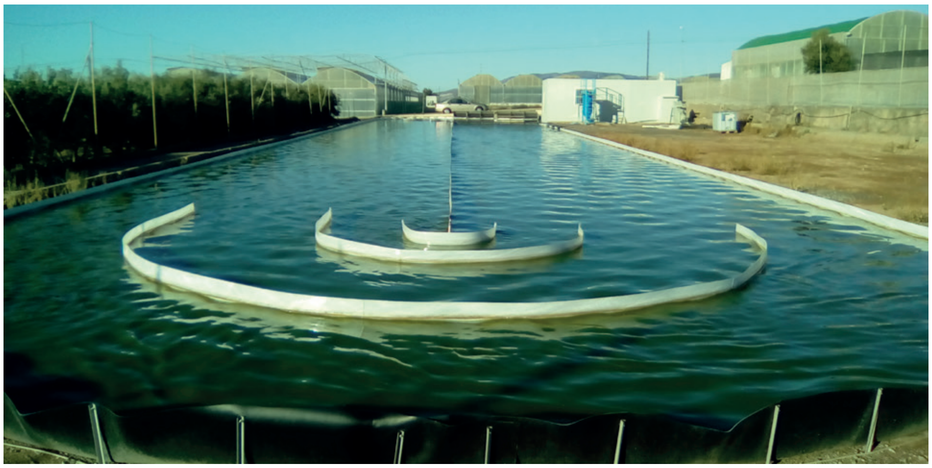

Image 1. Outdoor raceway reactors for microalgae biomass production located at the IFAPA facilities near the UAL campus

We are monitoring a series of pilot-scale microalgae photobioreactors to detect and investigate how microbial pathogens and diseases, commonly referred to as pests, spoil the microalgae production process. At WP1 scientists from different research organizations are in charge of managing and sampling the photobioreactors (Universidad de Almería, UAL), resolving the molecular formula of thousands of chemical compounds produced in the reactors, also known as chemical fingerprinting (Alfred Wegener Institute, AWI) and analyzing the taxonomic composition of the microbial communities accompanying (algal microbiomes) and disrupting microalgae (Consejo Superior de Investigaciones Científicas, CSIC). The ultimate goal of WP1 is to generate high-resolution time series of operational, chemical, and genomic data that allow us to reconstruct the time-varying interaction networks. The analysis of these time series will help uncover the triggers of catastrophic events (production crashes) in these industrially-relevant microbial ecosystems.

External raceways reactors

Since April 2021, two 80 m3 outdoor raceway reactors have been operating in continuous mode at the IFAPA facilities near the UAL campus. One reactor (image 1) is fed with municipal wastewater and the other with clean water supplemented with fertilizers. Both reactors have been intensively monitored during the last 9 months for the collection of i) real-time data (1 per minute) from the multiple pH, dissolved oxygen, and temperature sensors installed in the reactors and ii) discrete samples for chemical analysis and biological determinations. So far, we have collected more than 300 samples for chemical fingerprinting, 600 samples for genetic analysis (amplicon sequencing, metagenomics and metatranscriptomics) and many others for the determination of inorganic and organic nutrients, biomass concentration, dissolved organic matter fluorescence, etc. Once the data is processed and the time series is assembled, the potential of the data to produce new discoveries will be immense.

Bench-scale photobioreactors

Since September 2021, three 30-litre bench-scale photobioreactors have been monitored to investigate, under controlled light and temperature conditions, the most relevant chemical and biological changes preceding the failure of the microalgae biomass production process. (image 2). For this, the culture parameters such as temperature or pH in the photobioreactors are gradually increased until they exceed the thermal or pH tolerance range of the target microalgal species. We hypothesise that as temperature or pH approaches species tolerance limits, the performance of the culture will begin to show statistical signals, such as higher variance, slowdown recovery or flickering. These signals have been traditionally used as early warnings of system failure. Besides investigating these statistical signals, PRODIGIO aims to reconstruct the time-varying interaction networks. These networks, describing the interaction between the different biological and chemical components that make up ecological communities, are at the core of ecosystem functioning, and thus, their characterization and analysis are critical to deciphering the mechanisms underlying the failure of the system. Both, outdoor and indoor reactors are inoculated with the green microalgae *Scenedesmus*, but consistently, the cultures are rapidly invaded by hundreds of microorganisms, some beneficial and some detrimental. Exploring the effects of this amazing diversity on the production of microalgae biomass is one of the

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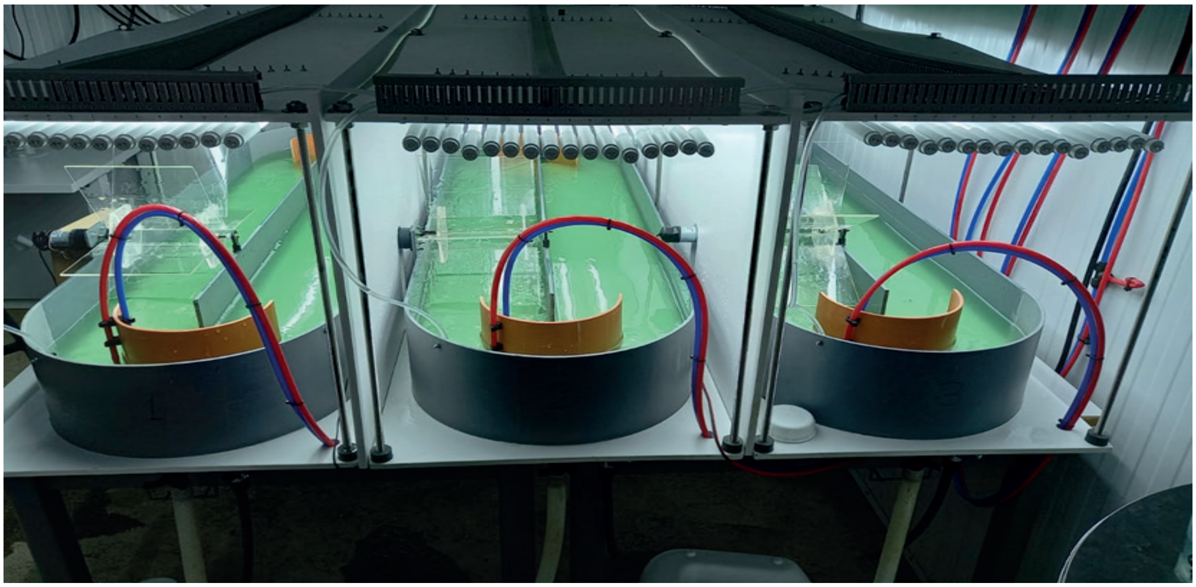

Image 2. Bench-scale photobioreactors located at the IFAPA facilities near the UAL campus.

most prominent objectives of the PRODIGIO project. If successful, PRODIGIO will contribute to filling a research gap that currently hinders the expansion of microalgae biomass production for commercial purposes.

First analisys

To date, more than 200 DNA samples have been sequenced for 16s (prokaryotes) and 18s (eukaryotes) and the sequences are currently being processed at CSIC to generate the first data tables (expected delivery date by March 2022). Our initial sequencing analyses, carried out in September 2021 to check the suitability of the sampling protocols and extraction procedures have been successful and show that outdoor reactors have an amazing microbial diversity. Likewise, chemical fingerprinting analyses are currently underway at AWI and their subsequent analysis using UltraMassExplorer - a browser-based application for the evaluation of high-resolution mass spectrometric data, will generate a database of thousands of chemical compounds unprecedented in the study of industrially relevant microalgae. Along with these two core sets of samples and analyses, scientists at UAL, AWI and CSIC are analysing many other samples for the determination of routine chemical and biological variables.

News on these analyses and the first results from the indoor reactors will be presented soon.

Our team

F. Gabriel Acién

Dr. Gabriel Acien, chemical engineer and coordinator of the biotechnology of microalgae group at the Universidad de Almeria, is the leader of WP1 and is responsible for project management and design of experimental plans.
facien@ual.es

Ana Sánchez-Zurano

Ana Sánchez-Zurano is a PhD student at the Chemical Engineering Department of the Universidad de Almeria. She is responsible for the management of photobioreactors and the modelling of results.
asz563@ual.es

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Martina Ciardi

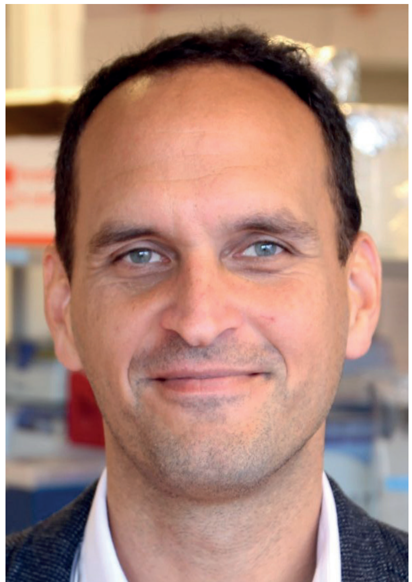
Martina Ciardi is a PhD student at the Universidad de Almeria, in Industrial Bioprocesses. The lines of research focus on the development of bioprocesses based on microalgae on a pilot and demonstrative scale, such as the treatment of agro-industrial waste and wastewater using microalgae

martina.ciardi@studio.unibo.it

Cintia Gómez Serrano

Cintia Gomez Serrano, degree in Marine and Environmental Sciences and PhD student at the Algae Biotechnology group of the Universidad de Almeria. She is responsible for the study of microalge cultures in seawater.

cgs1818@ual.es



Claudia Burau

Boris Koch

Claudia Burau is engineer in biotechnology. For 11 years she has been working at AWI and is in charge of the DOC (Dissolved Organic Carbon) analysis for the project.

claudia.burau@awi.de

Dr. Boris Koch is a senior scientist and head of the biosciences division (since 2019) and head of the department of ecological chemistry (since 2017) at AWI. His main interests include the biogeochemical cycling of marine dissolved organic matter and the role of chemical compounds in species interactions.

In PRODIGIO, he is the leader of the chemical fingerptining tasks

boris.koch@awi.de

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Failure tests in anaerobic reactors (WP2)

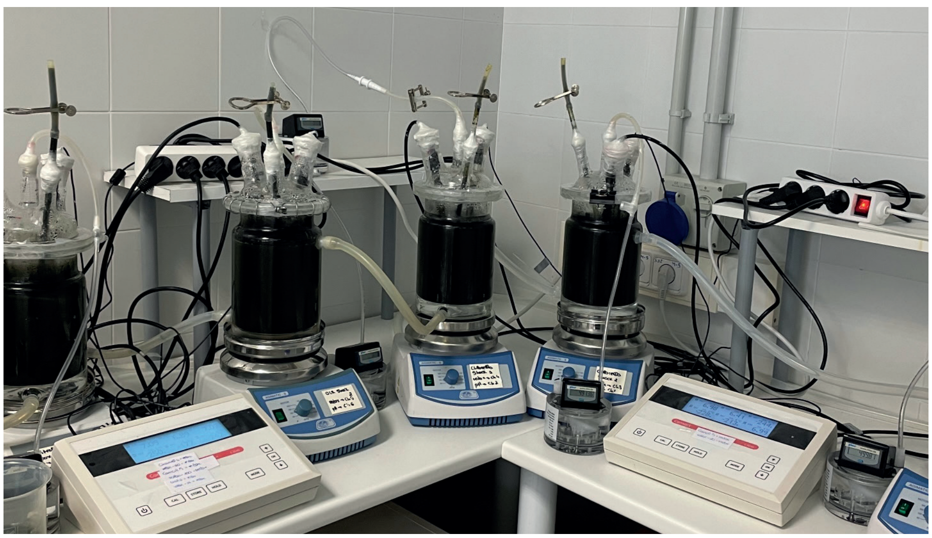

Image 3 Bench-scale anaerobic digesters located at the IMDEA-energy facilities

The overall goal of WP2 is to generate time-series data from perturbation experiments in continuous-flow anaerobic reactors (ARs) for subsequent identification of early warning signals, and quantification of threshold values and warning times. In the first nine months of the project, IMDEA Energy evaluated the microalgae anaerobic digestion failure against organic loading rate (OLR) disturbances. The experiments were conducted to identify the indicators that could provide early evidence of a biogas production decline. For such a purpose, 6 anaerobic reactors (ARs) were operated in parallel (Figure 3) using the microalgae *Scenedesmus* enzymatically pre-treated as feedstock.

Firstly, IMDEA-E has identified the best pretreatment prior to anaerobic digestion

The use of wastewater streams for microalgal biomass culture typically results in the growth of specific strains such as *Scenedesmus*, which possesses a rigid cell wall. This cell wall is composed of complex carbohydrates and is highly resistant to biological degradation. To enhance the microalgae bioconversion into biogas, and avoid uncontrolled instabilities, the proper pretreatment should be identified. Out of the pretreatments tested, the use of a proteolytic cocktail supported the best results in terms of biogas production. Thus, this pretreatment has been selected to favor the hydrolysis stage of anaerobic digestion.

Secondly, IMDEA-E has investigated the impact of high and low organic loading rates (OLR) shocks when using protein-rich microalgae biomass

The microalgae biomass was supplied by the partner UAL (Spain). Two controls were continuously running, while to study the effect of OLR shocks, four more digesters were operated. In this latter case, two of them operated at suboptimal OLR and starvation while the other two were fed with an increase of 4.6-fold the OLR of that of the control. The results demonstrated that while the ones subjected to low OLR shocks recovered right after the shock was stopped, the same was not true in the case of the reactors operated at high OLR shocks. In these later scenarios, intermediate metabolites of digestion accumulate rather than being consumed by methanogens. Anaerobic reactors subjected to high OLR shock required longer periods for process recovery (3 months). Overall, these results demonstrated the rapid response of the process to typical feeding inconvenients related to pumping problems in conventional facilities. In September 2021, IMDEA sent biological samples to the partners located at NMBU (Norway). The samples will be evaluated to establish a relationship between chemical and biological results upon OLR shocks. Indeed, NMBU is in charge of exploring the anaerobic microbial population dynamics involved in microalgal biomass biomethanization upon determined perturbations.

Our team

Cristina González-Fernández

Dr. Cristina González-Fernandez is the Head of the Biotechnology Unit of IMDEA Energy. Within PRODIGIO, she is leading WP2 and is responsible for project management, the design of experimental plans, and data analysis.
cristina.gonzalez@imdea.org

Elia Tomás-Pejó

Dr. Elia Tomás-Pejó is a Senior Assistant researcher at the IMDEA-E Institute. She has wide-ranging experience in the use of yeast for the production of biofuels and biochemicals from organic residues.
elia.tomas@imdea.org

Silvia Greses Huerta

Dr. Silvia Greses Huerta is a postdoctoral researcher at IMDEA Energy. Within PRODIGIO, she is responsible for the anaerobic digester operation and data analysis.
silvia.greses@imdea.org

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Modeling the Failure of Microalgae Production (WP3)

WP1 is generating an unprecedented 9-month time series of chemical and omics data with enormous potential to generate new insights and advance our current understanding of how to improve the performance of industrial-scale microalgae production systems. This objective requires a comprehensive analysis of how the different components of microalgae production systems, both chemical and biological, interact with each other and, more importantly, how this network of interactions changes over time. Thus, once the microalgae production time series data is assembled, a milestone expected for mid-2022, the project will move to the next stage: WP3 - Modeling the failure of microalgae production.

The overall goal of WP3, led by CSIC, is to analyse the mechanisms underlying the productivity decreases in microalgal PBRs, identify early warning signals, and evaluate, from the whole set of identified signals, those that meet the specified criteria of scalability, reliability, and affordability. CSIC scientists, in collaboration with computational ecologists at National Taiwan University, will analyze time series data using the most advanced methods developed to date for the reconstruction of interaction networks.

In particular, we will apply Empirical Dynamic Modeling (EDM) methods. EDM is an emerging data-driven framework for modeling nonlinear dynamical systems, such as ecological systems. At each time step, EDM computes the topology and strength of interactions of a time-series data interaction network. The time-varying interaction network estimates the stability of the system over time and allows the identification of the main contributors to process failure. In WP4, we will carry out an unprecedented analysis of interaction networks (interactomes) in microalgae photobioreactors with the ultimate goal of identifying early warning signals of system failure.

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Our team

Pedro Cermeño

Dr. Pedro Cermeño is a Senior Researcher at the ICM-CSIC in Barcelona, Spain. He is the project coordinator and he is in charge of project management and contributes to data analysis in WP3.
pedrocermeno@icm.csic.es

Ramiro Logares

Dr. Ramiro Logares is a Senior Researcher at the ICM-CSIC in Barcelona, Spain. He is the leader of WP3 and contributes to bioinformatics and molecular data analyses.
ramiro.logares@icm.csic.es

Celia Marrase

Dr. Celia Marrase is a Senior Researcher at the ICM-CSIC in Barcelona, Spain. She is in charge of analysing the fluorescence signals from microalgal photobioreactors.
celia@icm.csic.es

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Ramon Massana

Dr. Ramon Massana is a Senior Researcher at the ICM-CSIC in Barcelona, Spain. In PRODIGIO, he will contribute to molecular data analyses.
ramonm@icm.csic.es

Carles Pedrós-Alió

Prof. Carles Pedrós-Alió is a Research Professor at CNB-CSIC in Madrid, Spain. He has a strong interest in microbial diversity, the ecology of aquatic microorganisms, adaptations to extreme environments, and nutrient acquisition strategies by bacteria.
cpedros@cnb.csic.es

Carmen García-Comas

Dr. Carmen García-Comas is the project manager of PRODIGIO. She will also be involved in WP3 and WP4 due to her expertise on analysis of complex ecological datasets.
cgcomas@cmima.csic.es

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Caterina R. Giner

Dr. Caterina R. Giner is hired as lab technician for PRODIGIO. She is in charge of characterising the microbial communities by analyzing
genomic, flowcytometry, and organic matter samples.

caterina@icm.csic.es

Maria Montserrat Sala

Dr. Montse Sala is a Senior Researcher at the ICM-CSIC in Barcelona. Her role in PRODIGIO is to characterize algal microbiomes in photobioreactors.

msala@icm.csic.es

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Chih-hao Hsieh

Prof. Chih-hao Hsieh is professor at the NTU Institute of Oceanography. He is one of the developers of the Empirical Dynamic Modeling (EDM) for causal detection and other cutting-edge techniques for quantifying interaction networks in nonlinear dynamical systems. In PRODIGIO, he is the leader of the tasks related to EDM for early warning signals indentification.

chsieh@ntu.edu.tw

Modelling failure of anaerobic digestion (WP4)

As in WP1, our partners in charge of WP2 are generating an unprecedented time series of chemical and omics data from anaerobic reactors. Like WP3, WP4 – Modelling the failure of anaerobic digestion aims to reconstruct the biochemical and ecological interaction networks, which are at the core of ecosystem functioning. A thorough analysis of how these interaction networks, that is, their topology as well as the strength of the interactions change over time is crucial to predicting why, how, and more importantly, when the system starts to show warnings that a failure is about to happen. Anticipating the failure of the systems is the best way to improve their performance in the long term. WP4 is expected to start in mid-2022 once all bioinformatic analyses are completed and the data is ready for time series analyses.

WP4 is led by the Norwegian University of Life Sciences (NMBU) and, incollaboration with computational ecologists at National Taiwan University, will analyze time series data using Empirical Dynamic Modelling (EDM), one of the most advanced methods developed to date for the analysis of non-linear dynamic systems and reconstruction of causal interaction networks. Like in WP3, the application of EDM to time series of chemical and biological data collected in our bench-scale anaerobic reactors will help us uncover the mechanisms underlying the failure of these biogas production systems.

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Norwegian University of Life Sciences

Magnus Ø. Arntzen

Juline Walter

Dr. Magnus Arntzen is a researcher at NMBU in Oslo, Norway.He is in charge of WP 4 and his work is related to omics analysis, using quantitative techniques to retrieve relative expression levels in large datasets from both single species and complex communities.
magnus.arntzen@nmbu.no

Dr. Juline Walter is a Postdoctoral Researcher at NMBU, Ås. She is involved in WP2 and WP4 and aims to characterize the anaerobic communities over time and their major metabolisms
juline.walter@nmbu.no

Norwegian University of Life Sciences

Live Heldal Hagen

Phillip Pope

Dr. Live H. Hagen obtained her Ph.D. in microbial ecology of anaerobic systems, and is currently working as a research scientist and young PI (Young Research Talent grant funded by The Re-search Council of Norway) at the Norwegian University of Life Sciences - NMBU. Live is involved in WP2 and WP4.
live.hagen@nmbu.no

Dr. Phillip Pope is a Professor in Microbiology at NMBU in Oslo, Norway. His research interests seek to combine analytical metadata with metabolic reconstructions of population genomes to visualize flow of metabolites in complex microbiomes that inhabit digestive ecosystems.
phil.pope@nmbu.no

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Sustainability assessment (WP5)

The overall goal of WP 5, led by ARMINES-, is to evaluate and compare the potential environmental, economic, and social impacts of energy production from microalgal biogas with and without the early-warning technologies developed in PRODIGIO and to identify the most sensitive aspects to be considered for future commercial application. To do so, global sustainability is assessed from a life cycle perspective to account for the effects across the whole biogas production chain.

Environmental life cycle assessment

Environmental life cycle assessment (LCA) is being applied according to the 4 phases of ISO 14040 and 14044 standards (2006), namely the goal and scope definition, inventory analysis, life cycle impact assessment, and interpretation. The activities conducted to date have mainly focused on the goal and scope definition and the inventory analysis phases. ARMINES started data collection on both cultivation and anaerobic digestion stages to identify the process stages and input and output flows within the system boundaries and to build the life cycle inventories. To do so, ARMINES prepared two models of surveys, for microalgae production and anaerobic digestion, respectively. The process was enriched through discussions with external experts, such as the BIOCORE research team at Villefranche Oceanographic Observatory, in the South of France. In addition to the quantification of input and output flows, LCA modelling also requires estimates of the biomass and biogas production rates. A dynamic model is currently under development to assess the improvement of early warning systems compared to systems without this component. To this end, ARMINES has been studying existing models, mainly developed in Matlab, and the adaptations needed to be implemented in a compatible environment with available LCA tools (i.e. Brightway2 and lca_algebraic, in the Python language) in order to apply them to the PRODIGIO case study. The next steps will involve the application of the model to obtain the environmental LCA results for a selection of relevant impact categories.

Social life cycle assessment

The literature review to define the scope of the Social LCA is also ongoing. Two possible approaches are being considered: a reporting approach based on performance reference points (type I) and an impact pathway approach based on the identification of cause-effect chains (type II). The advantages and drawbacks of each approach are being analysed so as to select the most suitable approach for the PRODIGIO context.

Economic assessment and life cycle sustainability

After completing the environmental and social analysis, an economic assessment will be conducted, using a process simulation tool to complement the experimental data. Finally, results for the three dimensions will be interpreted to provide conclusions and recommendations on the sustainability of the process.

Our team

Paula Pérez-López

Mélanie Douziech

Thierry Ranchin

Dr. Paula Pérez-López, WP 5 leader, is an associate research scientist at the center O.I.E. of MINES ParisTech-PSL/ ARMINES. She coordinates activities on the environmental impact assessment of renewable energy systems.
paula.perez_lopez@mines-paristech.fr

Dr. Mélanie Douziech will contribute to PRODIGIO with her expertise in life cycle assessment and the quantification of ecotoxicological impacts of chemicals on the freshwater environment.
melanie.douziech@mines-paristech.fr

Dr. Thierry Ranchin is a Full Professor and Head of the Center O.I.E. of MINES ParisTech—PSL/ARMINES, whose expertise includes the evaluation of renewable energy resources. He holds a PhD in Engineering Science from the University of Nice-Sophia Antipolis. His research fields include image processing, wavelet analysis, multiresolution analysis, and satellite data fussion applied to renewable energy source evaluation.
thierry.ranchin@mines-paristech.fr

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Andriamahefasoa Rajaonison

Raphaël Jolivet

Andriamahefasoa Rajaonison is a PhD student at the center O.I.E. He will address potential impacts of PRODIGIO production system and its benefits compared to a conventional system, including environmental, social and economic dimensions.
andriamahefasoa.rajaonison@mines-paristech.fr

Dr. Raphaël Jolivet is an IT engineer. His role in prodigio will be to facilitate the implementation of parameterized models in LCA and the analysis of the effects of variability and uncertainty in LCA results.
raphael.jolivet@mines-paristech.fr

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Communication, dissemination and exploitation (WP6)

WP 6 is being led by IDConsortium. The main goal of this work package is to make the results of the project visible to research organisations and other stakeholders through communication, dissemination, and exploitation actions such as: (a) disseminating PRODIGIO knowledge and results through internal and external communications among consortium partners, the European Commission, and stakeholders, and (b) managing the information generated by the project in a fair and transparent manner for the consortium while supporting adequate knowledge transfer to the stakeholders (e.g. research organisations, private companies, etc.). Managing the expected wealth of information generated in the project in a fair and transparent manner for the consortium, as well as facilitating adequate knowledge transfer to stakeholders Among the tasks that will be undertaken in WP 8 are providing a set of communication and dissemination activities to sustain the long-lasting visibility of the project, implementing a strategic plan and collaborating with other global partners and consortiums with similar interests. All activities, plans, and actions taken will be actively monitored and assessed for their effectiveness to ensure that internal and external stakeholders are well informed about the status of the project. A final conference will be held at the end of the project to communicate and disseminate the project's results. During 2021, several activities have been undertaken. Regarding the communication and dissemination activities, a strategic plan for communication and dissemination of project results has been delivered; the PRODIGIO project website was designed during the first quarter of 2021 and launched in March. Thanks to the close collaboration with PRODIGIO partners, the PRODIGIO website is becoming a large repository containing the main information and activities of the project. Social media profiles have also been created on You Tube, LinkedIn, Facebook, Twitter, and Instagram to inform the different audiences about the ongoing activities and events and the development of the project. The first project video is complete and will be distributed via various communication channels.

With respect to the exploitation and IPR management of project results, the following tasks and activities have been done or delivered during 2021.

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Our team

Macarena Sanz

Macarena Sanz received her Agricultural Engineer degree from the University of Politécnica in Madrid and her Executive Master in Business Administration from IESE in Madrid.

Macarena is the managing director of IDConsortium.
msanz@idconsortium.es

Álvaro Tamayo

Alvaro Tamayo is Project Manager of IDConsortium and a geologist who graduated from the Complutense University of Madrid. He is in charge of PRODIGIO's communication and dissemination activities.
atamayo@idconsortium.es

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