PROMICON

Harnessing the power of nature through productive microbial consortia in biotechnology – Measure, model, master

LEGACY BOOKLET

This project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000733. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Commission. Neither the EU nor the EC can be held responsible for them.

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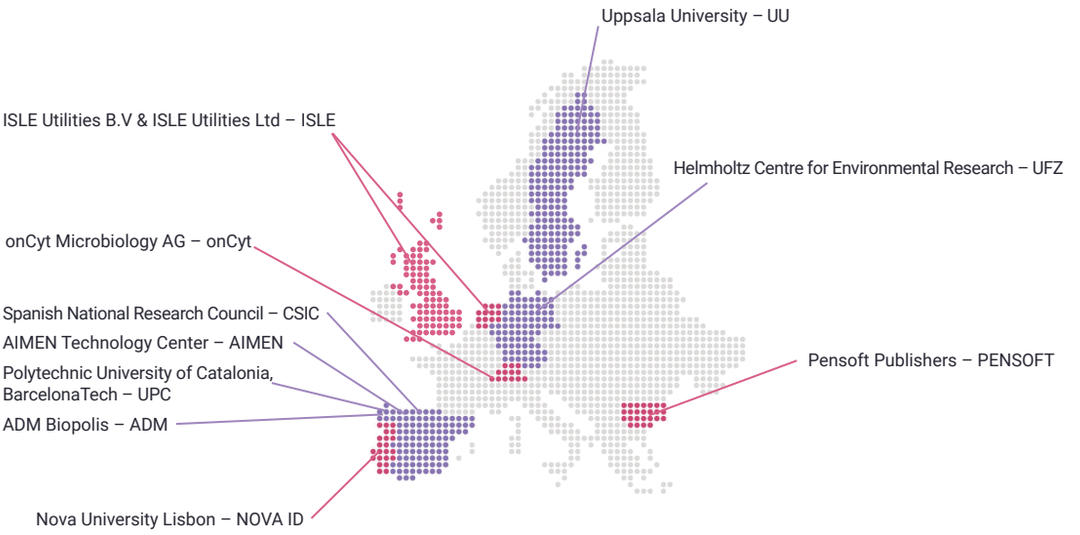

PROMICON Annual Meeting, 20–21 June 2022, Barcelona, Spain

PROMICON Annual Meeting, 3–4 July, 2023 - Lisbon, Portugal

PROMICON Annual Meeting, 28–29 May 2024 - Madrid, Spain

PROMICON

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Project overview

The overall objective of the PROMICON project was to learn from nature how microbiomes function in order to steer their phenotypes towards the production of biopolymers, energy carriers, drop-in feedstocks, and antimicrobial molecules. The project achieved this by developing novel analysis and modelling approaches that targeted key essential species in productive microbiomes, as well as whole microbiomes. PROMICON also established a standardised platform for obtaining quantitative single-cell data and connected coherent OMICS and Meta-OMICS data sets for complex microbiomes. The methodology involved a combination of data mining tools, mechanistic process models, as well as machine learning and deep learning approaches. The project employed synthetic biology and systems metabolic engineering to optimise and assemble bacterial farmers, producers, and stabilisers, providing optimal production of target metabolites. PROMICON focused on developing sustainable bioproducts that can contribute to the circular economy, thus aligning with the objectives of the EU 2018 Bioeconomy Strategy.

Project name

Harnessing the power of nature through **PRO**ductive **MI**crobial **CON**sortia in biotechnology – Measure, model, master

Duration

1 June 2021 – 31 May 2025 (48 months)

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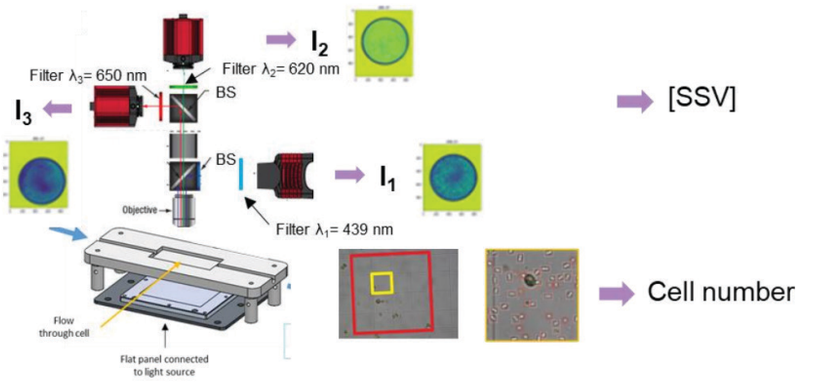
Work packages

WP1 Learning from nature – Enabling technologies

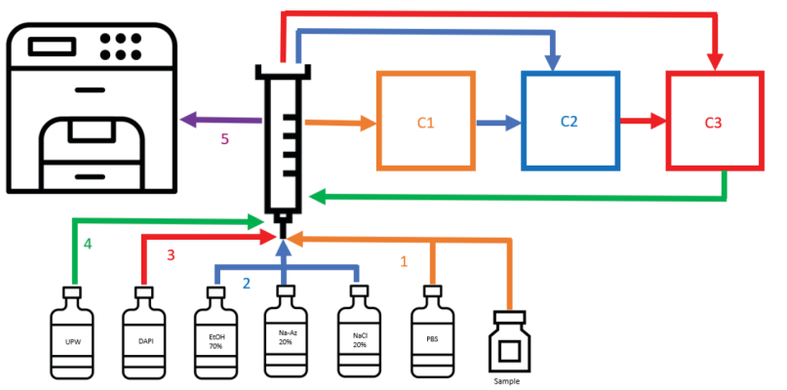
The ultimate goal of WP1 was to harness productive microbial consortia through learning from nature. Understanding how the individual strains in a consortium behave and deciphering the complicated interactions among strains is the key to overcoming the bottlenecks of such microbial consortium-based technologies. Therefore, WP1 developed comprehensive tools and a platform for understanding microbial consortia. This includes a flow-cytometry-based online monitoring system for cell quantification, a multi-omics-based platform for analysing metabolic activities, a Partial Least Squares model for predictions, data mining tools for interpreting community dynamic and cell functional changes, and applying machine learning and deep learning approaches to predict microbiome processes.

1.1 Development of an online microbial analysis platform

A defined online quantification and identification method for cell concentrations and heterogeneous cell states and types was developed using automated flow cytometry. To achieve this, a combination of an OC-300 automation system coupled with a DAPI staining and analysis process were designed. Furthermore, a developed hyperspectral monitoring system was achieved that enables the quantification of biomass, pigment, and PHB. This system was applied to monitor biomass and PHB production in real samples from partners.



Multispectral sensor for biomass estimation. Second configuration with simultaneous acquisition mode.



Scheme representing the workflow of automated dilution, fixation, staining and measurement. **1.** The sample is diluted 1:2 with PBS and moved to an intermediate container called chamber 1 (C1). **2.** The sample is moved from C1 to chamber 2 (C2) and fixed with NaCl 20%, Na-Azide 1% and EtOH 10% for 10 min. **3.** The fixed sample is moved from C2 to chamber (C3) and stained with DAPI 1 mM for 10 min. **4.** The stained sample is diluted 1:20 with ultra-pure water and, **5.** The diluted sample is sent to the flow cytometer to be measured.

1.2 Development of a microbial “omics analysis platform”

By combining proteomics, metabolomics, and stable isotope labelling techniques, a comprehensive omics analysis platform was developed aiming to understand individuals within complex microbial consortia. This platform was successfully applied for analysis of pure culture of engineered strains, coculture of phototrophs, and natural consortia. The results provided deep insight into microbial interactions and could thus help to improve efficiency in preparing final products.

1.3 Environmental microbiome database mining and contextualisation of selected microbiomes

A computational workflow was established to identify and measure the abundance of taxonomies within target microbiomes. Real environmental samples were collected and examined using the workflow to understand microbial composition.

1.4 Hybrid modelling platform

A hybrid modelling platform based on Physics-informed neural networks for dynamic modelling and control of natural microbiomes was established. This modelling platform used the deep learning method of adaptive moment estimation and was applied further for automatic control of a bioreactor.

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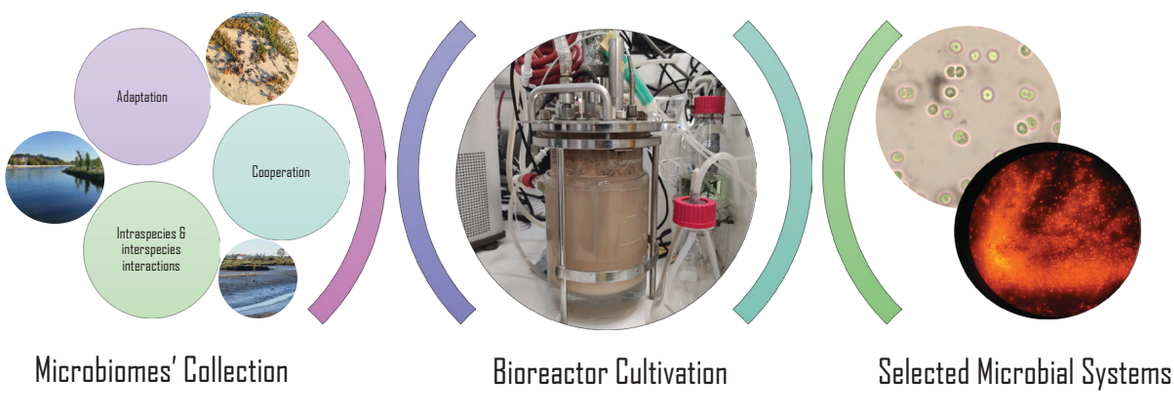
WP2 Natural consortia for learning and production

Natural microbiomes, given their high ecological and metabolic diversity, can be explored to identify strains with unique biosynthetic capabilities, including the ability to synthesise polyhydroxyalkanoates (PHAs), extracellular polysaccharides (EPS), and/or phycobiliprotein pigments (PPP). PHAs or biodegradable plastics, are accumulated by many bacteria as internal energy reserves, often under nutrient-limited conditions. Similarly, polysaccharides, valued for their biocompatibility and functional properties, are derived from microbial sources like bacteria, cyanobacteria and microalgae, offering applications in pharmaceuticals and food industries. Pigments from microorganisms provide eco-friendly alternatives to synthetic dyes, with potential uses in textiles, cosmetics, and food. By leveraging advanced biotechnological tools, these microbial systems can be optimised, enhancing yield and sustainability while reducing reliance on traditional chemical processes. This approach not only supports a circular bioeconomy but also addresses environmental challenges.



2.1 Collection, pre-screening and characterisation of natural microbiomes

Several environmental samples from different geographical areas, covering a large diversity of habitats (e.g., marshland, forest soil, river sediments, plant roots, constructed wetland, and urban ponds) were collected and characterised in terms of their physicochemical and morphological properties with the aim to identify those with ability to synthesise the target products - PHA, EPS and/or PPP.

2.2 Microbiome development by ecological selective pressure

The selected microbiomes were subjected to selective pressures for enrichment in microorganisms capable of producing PHA, EPS and/or PPP. Advanced biomolecular techniques based on 16S rRNA gene amplification were used to validate the selective pressure applied to the collected field environmental samples and to identify microorganisms in the evolved microbiomes. The obtained bioproducts were characterised.

2.3 Bio-production by controlled microbiomes

The best-performing microbiomes are being cultured in production bioreactors to maximise yield and efficiency. The simultaneous production of PHA and EPS by cyanobacteria was explored, and a scale-up experiment was conducted using a 3-liter photobioreactor (PBR) with a dual-cycle approach. For PHA production by heterotrophic organisms, a Physics-Informed Neural Network (PINN) approach was used for the first time to define the optimal set of control parameter values towards maximum PHA production by a microbiome evolved from marshland river sediments. An EPS producer was isolated from the same natural microbiome and, upon bioreactor cultivation, it reached a high production of an EPS rich in glucosamine, presenting gelling capacity and the ability to form/stabilise emulsions with almond, olive and sunflower oils.

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WP3 Synthetic consortia for production

WP3 used a bottom-up approach to design synthetic consortia for the targeted production of PHACOS (an antimicrobial polyhydroxyalkanoate (PHA) derivative), butanol, and H_2, from CO_2 (carbon source) and light (energy source). These communities are much simpler than natural ones and include organisms that have been engineered for the production of target molecules. In these consortia, metabolic engineering is being used to (i) introduce the production pathways of interest not found in natural strains, and (ii) implement the necessary control measures (e.g., removing competing pathways, enzymatic bottlenecks, etc.) for adjusting the consortium to the desired phenotypes.

3.1 A synthetic phototrophic microbial consortium for sustainable bioenergy production

The biosynthesis of acetate from CO_2 and light was optimised through metabolic engineering of the cyanobacterium *Synechocystis* PCC 6803. Insertion of a phosphoketolase (PK) in the *acs* gene resulted in an enhanced Calvin-Benson-Bassham cycle and 40-fold higher acetate production. Further overexpression of a phosphotransacetylase (Pta) led to an 80-fold increase, reaching an acetate production of 2.3 g/L. A synthetic consortium, based on the *Synechocystis* PCC 6803 strain that secretes acetate and a phototrophic bacterium *Rhodopseudomonas palustris* growing on the formed acetate, enabled the production of biohydrogen and fatty acids through nitrogen and carbon dioxide fixation. Elemental balance confirmed carbon capture and nitrogen fixation into the consortium. Proteomic analysis indicated acetate exchange and light-dependent regulation of metabolic activities.

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3.2 Optimisation of a sucrose overproducer *Synechococcus* strain

We aimed to obtain a superior Farmer strain providing a high level of sucrose from CO_2 and light as feedstock for Labour heterotrophic modules. Starting with the sucrose overproducer *Synechococcus* strain SBG363 (Patent WO 2021/148693 A1), we designed a further optimisation strategy, by applying strain-designing algorithms and the metabolic model of *Synechococcus i*JB7852, based on deleting key genes involved in sucrose competing pathways. A total of three genes were identified and deleted in wild-type *Synechococcus*, which cause growth impairments, especially the glycogen synthase knock-out (Δ*glgA*). Finally, the sucrose overproducing strategy was implemented in the triple mutant and sucrose production was assayed. Preliminary results indicated that a deeper understanding of sucrose and glycogen metabolism in *Synechococcus* is needed to further optimise sucrose production.

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3.3 An optimised *P. putida* biocatalyst for the sustainable overproduction of antimicrobial bioplastics (PHACOS)

Using a rational, model-driven metabolic engineering approach, we have designed a *Pseudomonas putida* KT2440 biocatalyst capable of efficiently utilising sucrose produced by cyanobacteria from CO_2 and light while enhancing acetyl-CoA levels. These metabolic improvements enable the seamless conversion of acetyl-CoA into bioplastics – specifically, medium-chain-length polyhydroxyalkanoates (PHAs) – without nutrient limitation constraints. Supplementing the growth medium with 6-acetyl-thiohexanoic acid (6-ATH) facilitates the accumulation of up to 70% (g/g CDW) of a high-value functionalised PHA, known as PHACOS, which exhibits antimicrobial activity against pathogens such as *Staphylococcus aureus*.

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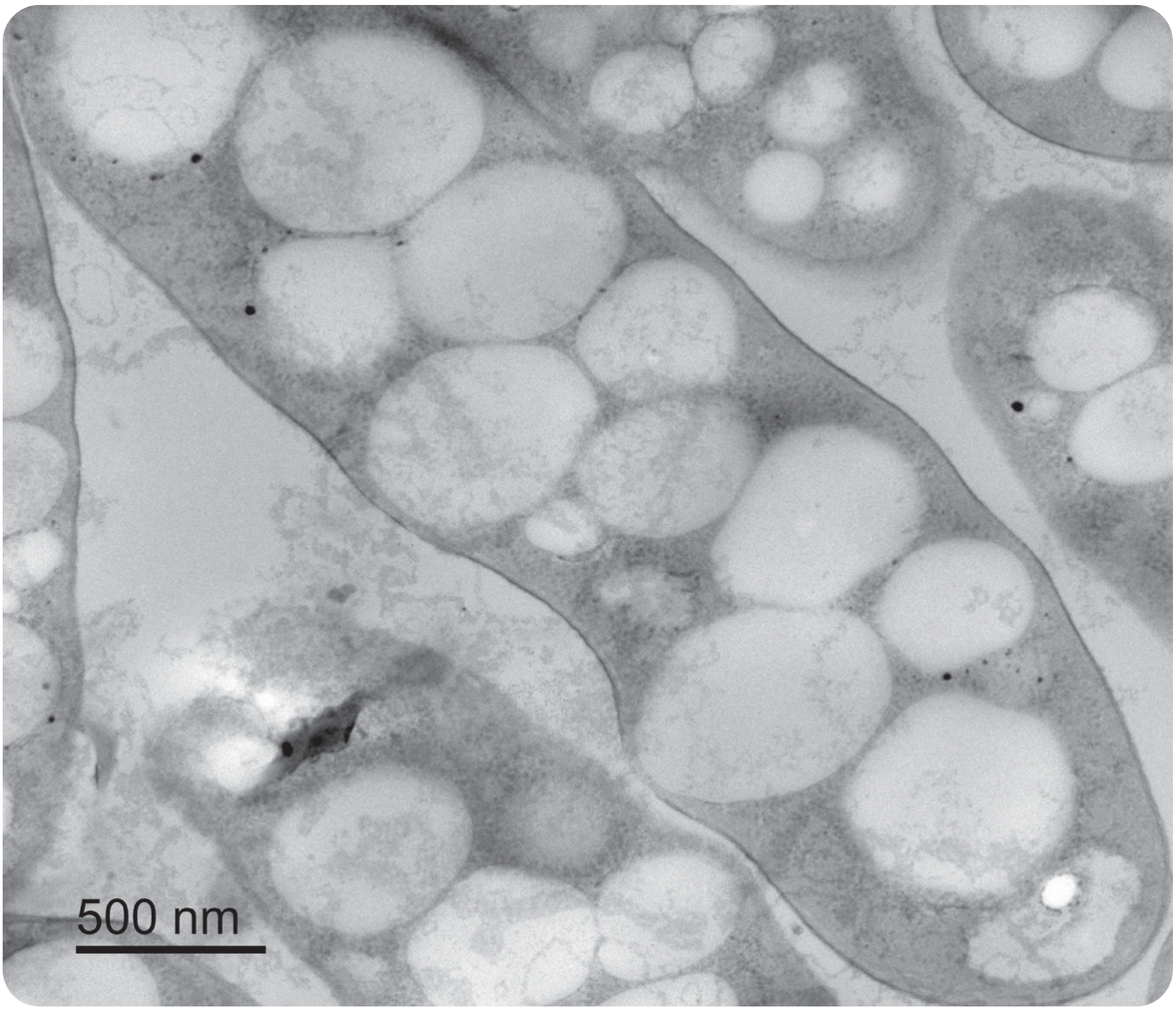


Pseudomonas putida biocatalyst accumulating PHACOS.

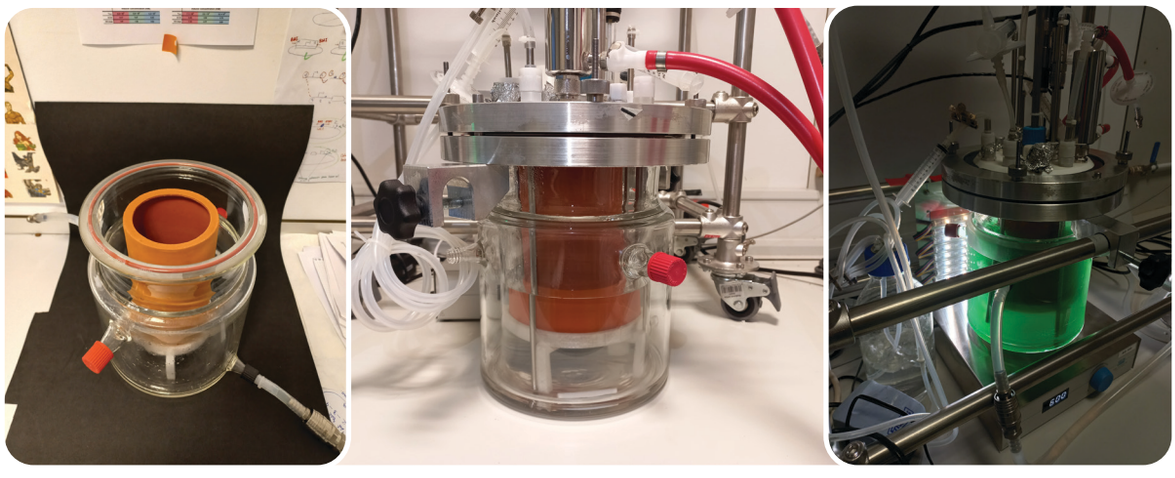
WP4 Reactor concepts

Microbiome applications face challenges due to a lack of suitable reactor systems, especially for phototrophic organisms. Current low-tech systems limit biomass concentration, reducing productivity and product yield. To compete with specialised fermentation systems, new reactor technologies enabling continuous operation and high space-time yields are essential.

4.1 Development of a multi-chamber lab-scale photobioreactor suitable for microbiome-based bioprocesses

A tailored compartmentalised photobioreactor was designed using a division of labour at phenotype and spatial levels. Unlike prior small-scale approaches, this is among the first reactor-level implementations. Validated with ceramic membranes for controlled metabolite diffusion, it enables co-cultivation of heterotrophic and autotrophic organisms for high-value bioproduction. This multi-chamber design accelerates microbial consortium analysis, offering insights for system simulation and optimisation.

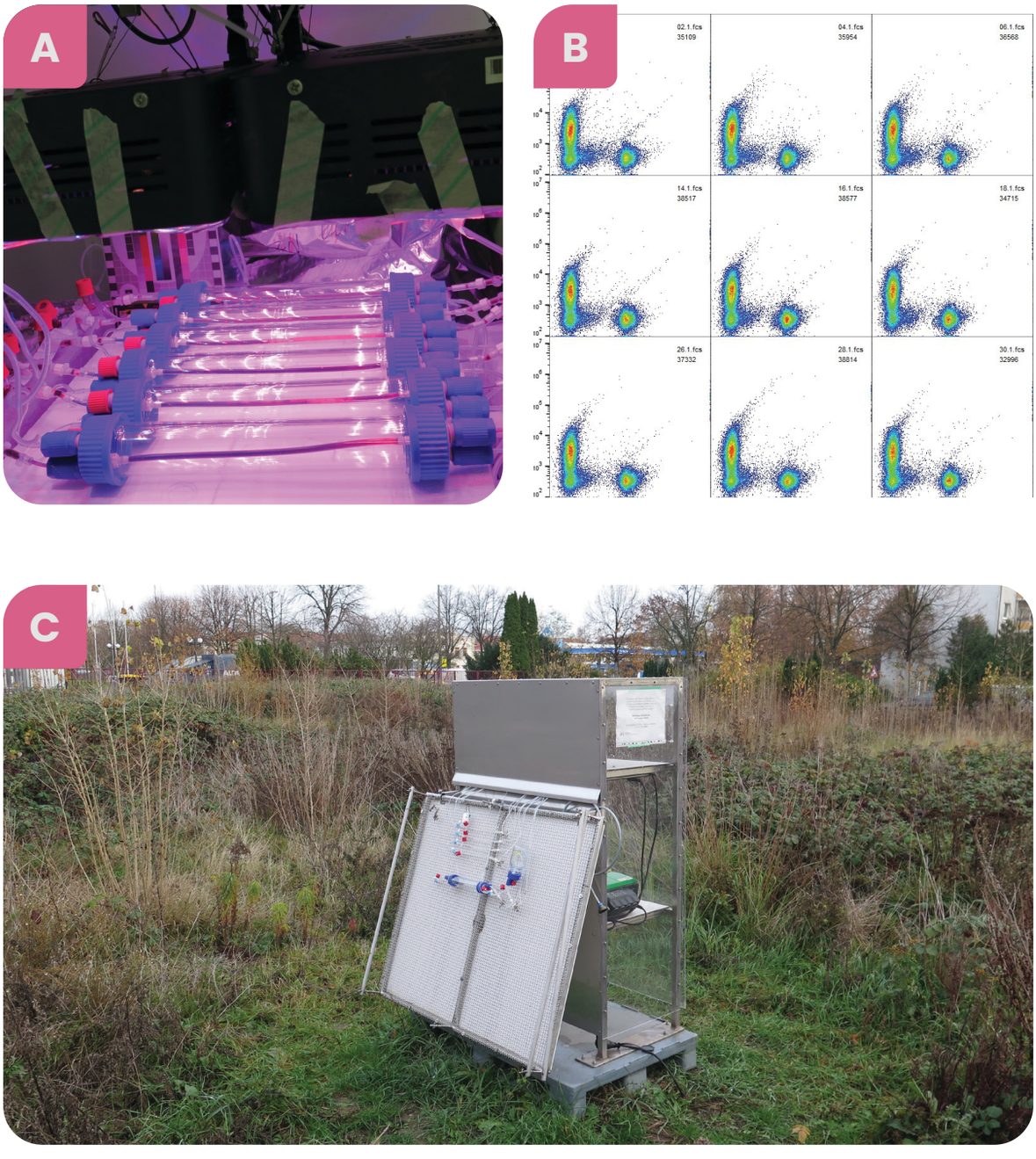
A corresponding model integrates CFD and biological modelling to simulate microbial interactions. Based on a modified BIO_ALGAE framework, it analyses members of the artificial microbial community in separate chambers, optimising conditions while enabling metabolic exchange. COMSOL Multiphysics™ models fluid dynamics, heat transfer, and species transport to predict optimal growth conditions.



Multi-chamber photobioreactor setup. The figure shows the compartmentalised bioreactor by an external light and a CO_2 farming chamber and an internal production ceramic chamber. Both spaces have independent culture settings according to the growing organism.

4.2 Development of a capillary biofilm reactor suitable for bio-film based bioprocesses

A gas-tight capillary bioreactor was developed to minimise product loss (H_2), while a cell cytometry-based method was established for real-time biomass composition assessment. Additionally, EPS production analysis provided insights into biofilm performance. Initial outdoor experiments highlighted key operational constraints for future optimisation.



Capillary biofilm reactor in the lab (A) and outside running under real-world conditions (C). (B) shows example graphs from flow cytometry allowing close monitoring of population dynamics in the reactor.

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WP5 Exploitation

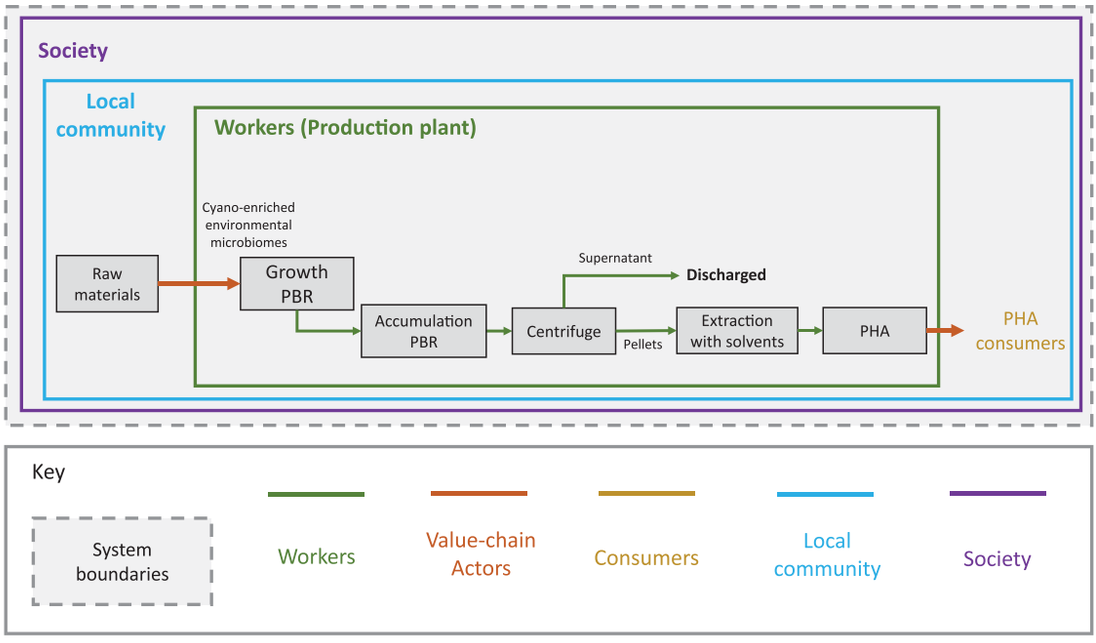
WP5 aimed to maximise the impact of PROMICON results, facilitate stakeholder engagement, and boost market uptake of project solutions. To achieve this, WP5 assessed the environmental, economic, and social impacts of target bio-based products to estimate the feasibility of large-scale production. It supported innovation activities by managing intellectual property, identifying and quantifying market opportunities and barriers, incorporating stakeholder feedback for further development, and developing exploitation strategies and business models with strong value propositions.

5.1 Impact assessment – Economic Analysis and Life Cycle Assessment

Within WP5, Life Cycle Cost (LCC) analysis and Life Cycle Assessment (LCA) of the products, obtained as a result of the novel processes and technologies developed throughout the PROMICON project, were performed. For these assessments, several scenarios to produce five products (PHA, EPS, PHB, pigments and hydrogen) in a more efficient, sustainable and viable way, currently in high demand by the industry, were considered in order to determine the economic and environmental viability of the implementation of these new technologies on a large scale. The PROMICON groundbreaking technologies are still in their early stages, offering great potential for the future. As a result, gathering large-scale data on various processes – (such as energy consumption, volumes, times, and equipment flows) – remains a challenge. However, this provides an incredible opportunity to refine and optimise these processes. While it is challenging to produce a precise LCC and LCA that reflects what would occur at an industrial scale, the progress made within WP5 lays a strong foundation for future advancements.

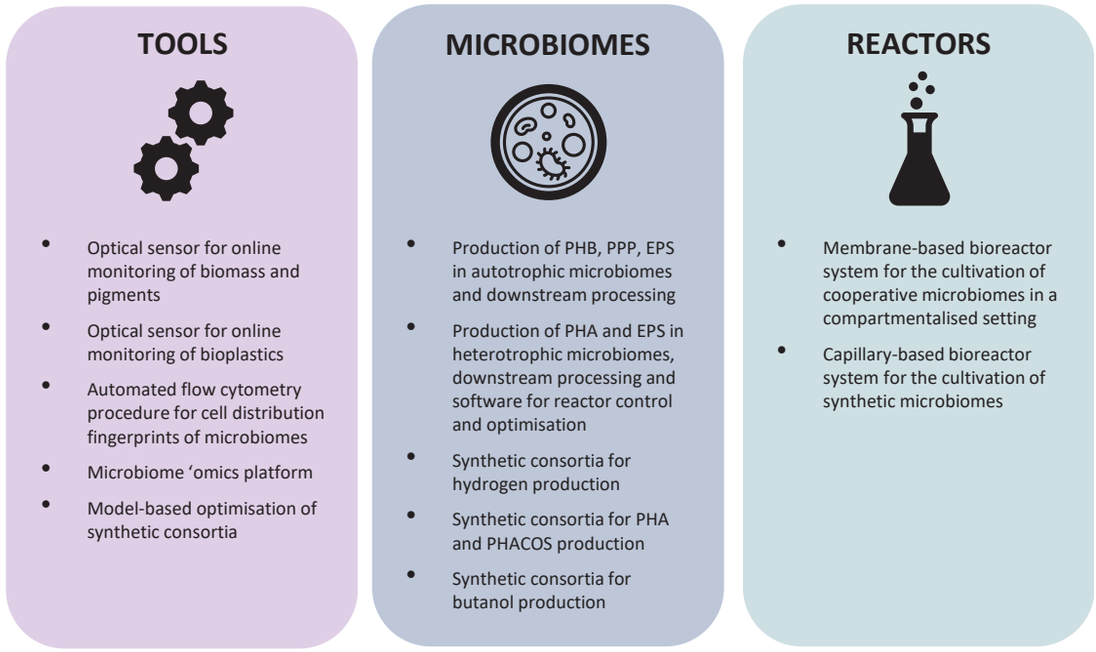
5.2 Impact assessment – Social Life Cycle Assessment

A Social Life Cycle Assessment was carried out to analyse the social performance of the production of four bio-based products for both food and non-food applications (i.e., additives, bioplastics, pigments and hydrogen) by means of novel bio-based routes based on microbiomes. The results pointed out that all of them showed good social performance, even though the sole production of bioplastics and hydrogen had the best overall social performance. This was mainly due to the high acceptance level for consumers and the better performance in terms of public commitment to sustainability issues for society. Indeed, the non-food products (i.e., bioplastics and hydrogen) seemed to have higher acceptability from consumers and higher interest in terms of regulation and policy development. More efforts should be made to develop specific regulation and policy. Also, implementation at full-scale should be boosted to cover the technological development gap.



5.3 Exploitation Strategy and IP management

To develop an effective and tailored Exploitation and Business Strategy Plan for the PROMICON project, the Key Exploitable Results (KERs) of the project – reported in the Figure below – were identified and for each of them, the Intellectual Property protection tools were defined. Based on the identified KERs, which are linked to the production of biopolymers, butanol and hydrogen, a market and competitor analysis was carried out to identify risks and opportunities for the exploitation of the PROMICON solutions. Moreover, to maximise the impact of the innovations developed during the project, key stakeholders and end-users were engaged and invited to three Innovation Workshops to provide feedback to further develop PROMICON solutions and ensure they are fit-for-purpose. The outputs of the market analysis and the Innovation Workshops helped refine the business strategies and exploitation roadmaps of the PROMICON solutions to boost their market uptake beyond the project.

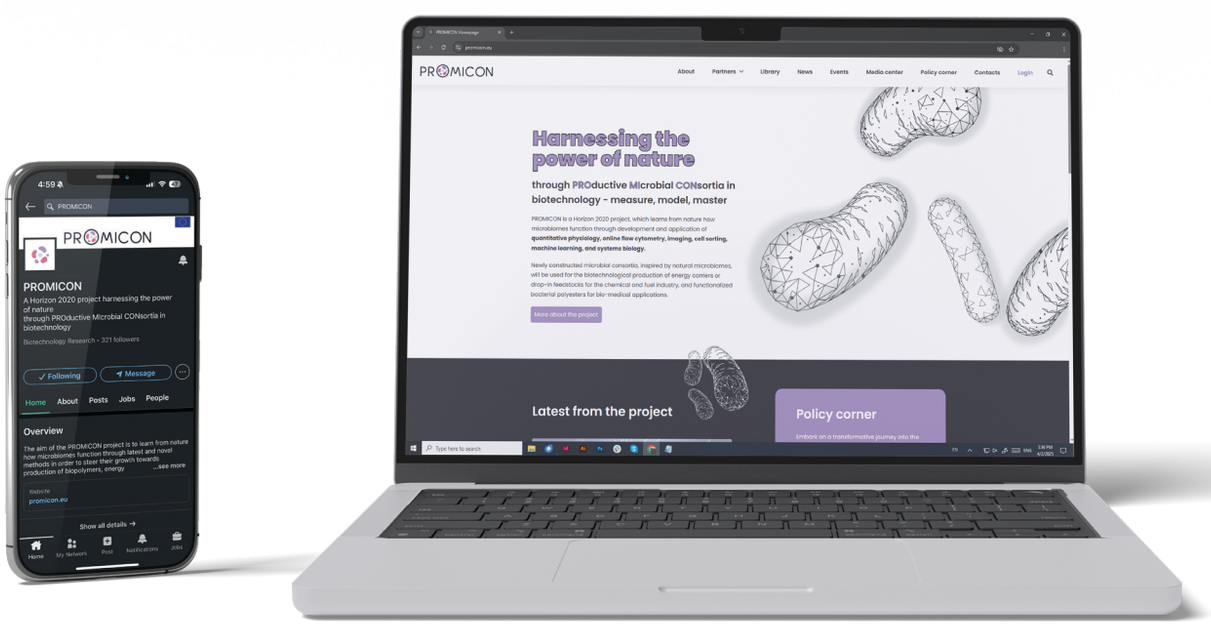


WP6 Dissemination and communication

WP6 focused on project branding, communication, and dissemination. Key tasks included setting up and managing the project's visual identity (logo, promotional materials, website, templates, social media) to ensure effective communication. The project's website (www.promicon.eu) served as a central hub for information and was regularly updated with content about the project's goals, activities, and results. We have also utilised various outreach platforms to disseminate PROMICON's results, including newsletters, press releases, and social media channels, to reach a wide and diverse audience. Additionally, we shared research outcomes in a targeted manner through platforms such as the Horizon Results Platform, the EU Knowledge Center for Bioeconomy and CORDIS, ensuring that our findings reach relevant stakeholders and are properly disseminated within the academic and industry sectors. Furhermore, our knowledge transfer strategy included developing targeted materials like graphical abstracts for key publications, policy briefs, tutorial videos and scientific posters to enhance dissemination.

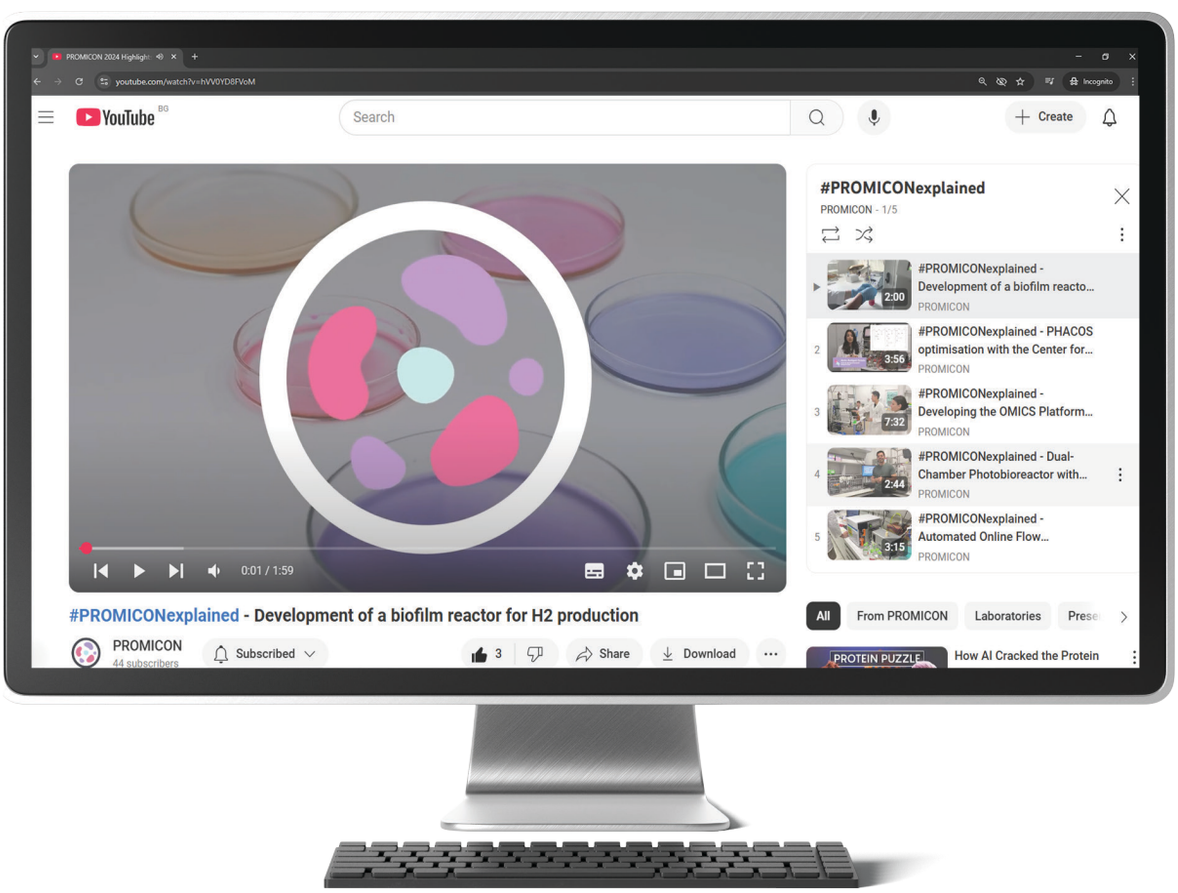
6.1 Policy briefs

PROMICON created several policy briefs that address key areas of scientific and technological advancements relevant to policy and practice. These briefs focus on strengthening the EU Bioeconomy Strategy through microbiome analysis, supporting the transition to a circular bioeconomy, and developing zero-emission processes for biodegradable plastics. The policy briefs are available in the "Policy Corner" on PROMICON's website, providing insights to inform decision-making and foster sustainable solutions.



6.2 Video content

To highlight the main technological achievements of the PROMICON project, videos were produced to showcase its key results. They focus on the project's scientific developments, presenting them in an accessible and engaging way. Aimed at making the project's work easier to understand, they demonstrate the potential scientific impact of the project. The videos are available on PROMICON's YouTube channel, where viewers can explore the project's outcomes in more detail.



6.3 Open-access project collection

The launch of the PROMICON RIO Collection in the Research Ideas and Outcomes (RIO) journal was a significant step for the project, offering a transparent and easily accessible record of its research progress. This open-access collection provides a comprehensive, ongoing view of PROMICON's developments, with research articles, preprints, policy briefs, deliverables, and project reports. Continuously updated, the collection reflects the project's advancement as new results emerge. By providing access to this collection through only one DOI link (DOI: 10.3897/rio.coll.239), all stakeholders can easily explore the full range of materials within the collection, gaining insights into PROMICON's activities.

Scan the Linktree QR to access PROMICON'S collection in RIO, as well as further information on the project.

References

PROMICON RIO collection: https://doi.org/10.3897/rio.coll.239
Graphical summaries: https://promicon.eu/media-center/graphical-summaries
Policy briefs: https://promicon.eu/media-center/policy-corner
Website: https://www.promicon.eu
PROMICON YouTube channel: https://www.youtube.com/channel/UCAI--XCOg0aR3ExisRdhVrQ
X/Twitter: https://x.com/promiconh2020
LinkedIn: https://www.linkedin.com/company/promicon

Graphical summaries

MONITORING PHB PRODUCTION IN *SYNECHOCYSTIS* SP. WITH HYPERSPECTRAL IMAGES

Key message

This innovative study highlights the transformative impact of hyperspectral technologies on optimising PHB production from cyanobacteria. The combination of hyperspectral imaging with advanced analytical techniques offers the potential to **revolutionise the management of wastewater treatment and promote the development of sustainable bioplastics.**

Background

Cyanobacteria possess the ability to accumulate polyhydroxybutyrates (PHB), a valuable material for bioplastic manufacturing. However, achieving optimal PHB production demands precise cultivation conditions and constant monitoring, posing a significant challenge for existing methods.

Objective

This study harnessed cutting-edge hyperspectral imaging technologies to track the growth of cyanobacteria and the production of bioplastics (PHB).

Impact

- The application of hyperspectral technology enabled meticulous monitoring of cyanobacteria populations and PHB production, offering crucial insights for **enhancing wastewater treatment processes and optimising bioplastic yield.**
- The research emphasised the importance of creating specialised sensors capable of adapting to different bioreactor scales affordably. It suggested that the integration of multispectral sensors with customised filtering mechanisms could streamline the process by replacing intricate hyperspectral systems. This simplification would facilitate their **incorporation into bioreactors of varying sizes.**

Results

As a collaborative effort between the research groups of AIMEN and UPC, an innovative measurement approach was devised capable of detecting subtle changes in the spectral reflectance of light emitted by cyanobacteria across different cultivation conditions and cellular stages. Leveraging hyperspectral images, the study successfully differentiated between cyanobacteria species within laboratory and pilot-scale bioreactors.

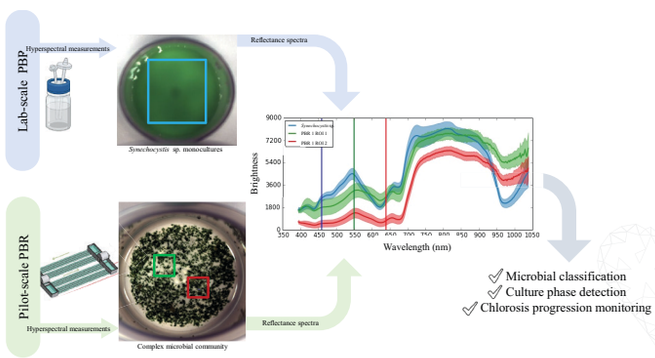


Figure. Comparison between reflectance spectrum of *Synechocystis* sp. from lab-scale PBR and spectra obtained in two different areas from pilot-scale PBR 1. (a) Image of a sample from *Synechocystis* sp. monocultures (top petri dish) and image of a sample from pilot-scale PBR 1 (bottom petri dish). Different selected ROI corresponding to *Synechocystis* sp. monoculture (blue square) and pilot-scale PBR 1 (green and red squares). (b) Mean reflectance spectra for lab-scale PBR containing *Synechocystis* sp. monocultures obtained during the growth phase and the two selected ROI for pilot-scale PBR 1 sample.

Source

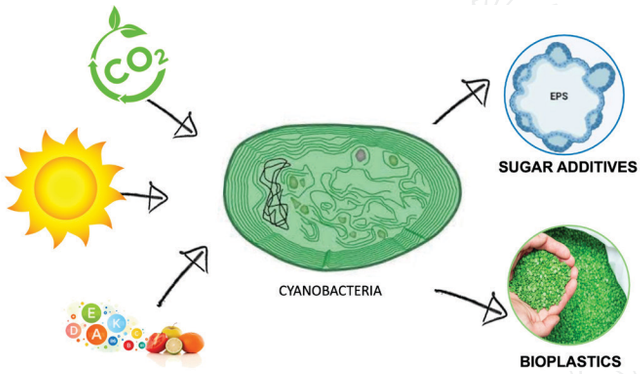
Rodríguez Lorenzo, F., Placer Lorenzo, M., Herrero Castilla, L., Álvarez Rodríguez, J. A., Iglesias, S., Gómez, S., Fernández Montenegro, J. M., Rueda, E., Diez-Montero, R., Garcia, J., & Gonzalez-Flo, E. (2022). Monitoring PHB production in *Synechocystis* sp. with hyperspectral images. *Water Sci Technol*, 86(1), 211–226. https://doi.org/10.2166/wst.2022.194

PROMICON

NEW STRATEGY FOR BIOPLASTIC AND EXOPOLYSACCHARIDES PRODUCTION: ENRICHMENT OF FIELD MICROBIOMES WITH CYANOBACTERIA

Key message

This study showcases the promising potential of harnessing the power of cyanobacteria for biopolymer production, offering a sustainable solution to the growing concerns surrounding plastic pollution and **paving the way for a more eco-conscious approach to material manufacturing.**



Background

Cyanobacteria are fascinating microorganisms that can thrive using sunlight and CO2, similar to plants and algae. What makes them even more intriguing is their ability to produce substances resembling plastic, known as bioplastics, or sugar additives.

Objective

This study delved into the realm of cyanobacteria, exploring their capacity to produce biopolymers as **eco-friendly alternatives** to conventional polymers. By **COLLECTING** samples from rivers and wetlands and creating lab conditions mimicking natural environments with nutrients, light, and CO2, researchers aimed to boost the **GROWTH** of photosynthetic microorganisms for bioproduct production.

Methods

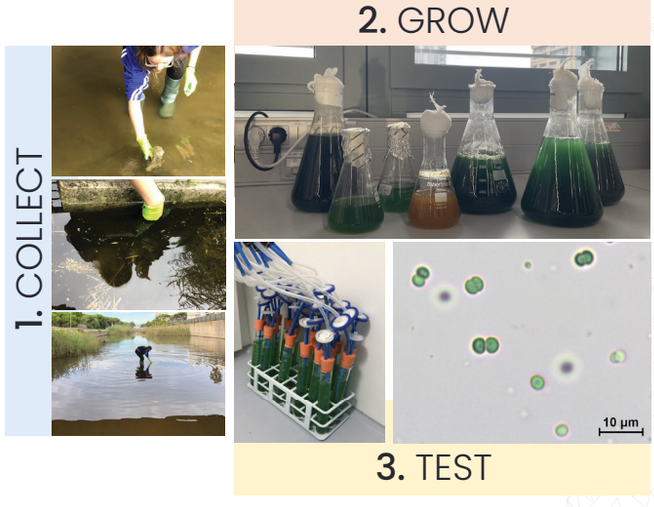
Initially, the goal was to explore methods for increasing the presence of cyanobacteria in these natural samples. After getting the cyanobacteria cultures, researchers **TESTED** their ability to synthesise both bioplastics and sugars. By supplementing with acetate, they found a positive impact on biopolymer production, paving the way for a **more sustainable approach to manufacturing bioplastics.**

Impact

- The study not only identified cultures with high bioproduct yields but also highlighted the potential for scaling up production in larger reactors. This breakthrough offers industries in **textiles, food and cosmetics** valuable insights into utilising cyanobacteria-enriched microbiomes for **sustainable biopolymer production.**

- By optimising culture conditions and nutrient supplementation, the study demonstrated the feasibility of enhancing bioplastic and sugar additive synthesis in an environmentally friendly manner.

- This research opens doors to a greener future, where bioplastics derived from cyanobacteria could serve as a **viable alternative to conventional plastics, reducing the environmental impact of plastic waste.**



Source

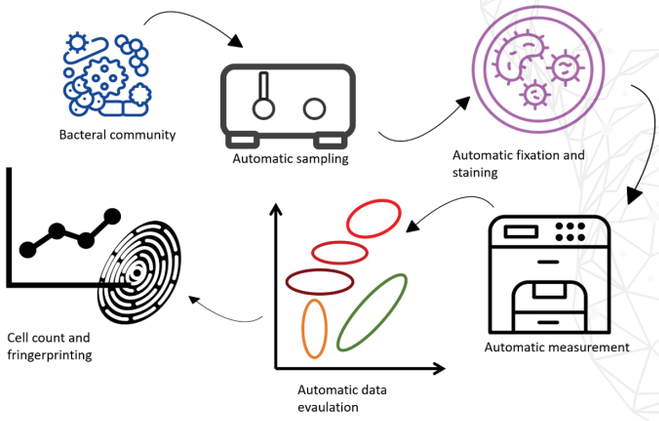
Altamira-Algarra, B., Rueda, E., Lage, A., San León, D., Martínez-Blanch, J.F., Nogales, J., García, J., Gonzalez-Flo, E. (2023). New strategy for bioplastic and exopolysaccharides production: Enrichment of field microbiomes with cyanobacteria, *New Biotechnology*, 78 https://doi.org/10.1016/j.nbt.2023.10.008. Now published in *Algal Research*: https://www.sciencedirect.com/science/article/pii/S1871678423000560

PROMICON

DEVELOPMENT OF AN AUTOMATED ONLINE FLOW CYTOMETRY METHOD TO QUANTIFY CELL DENSITY AND FINGERPRINT BACTERIAL COMMUNITIES

Key message

This method can reproducibly measure both cell density and fingerprint-like patterns of bacterial communities, generating suitable data for powerful automated data analysis and interpretation pipelines. The automated, high-resolution sorting of clustered data into cell subsets allows the identification of operational or abiotic/biotic causes of community disturbances or state changes. Such disturbances or changes can influence the interaction potential of organisms in microbiomes or even affect the performance of individual organisms.



Background

Cell density is among the most commonly used parameters for the operation and control of industrial biotechnological processes. In the past, its determination was often performed offline and manually, resulting in a delay between sampling and immediate data processing, which prevents quick action. While there are now some online methods for rapid and automated cell density determination, they are unable to distinguish between the different cell types in bacterial communities.

Objective

This study proposes an automated monitoring system comprising hardware, software and an automated workflow in order to enable real-time high-resolution analysis of bacterial communities. On the one hand, it allows for the online automated calculation of cell concentrations and, on the other, for the differentiation between different cell subsets of a bacterial community.

Methods

The OC-300 automation device (onCyt Microbiology, Zürich, Switzerland) was coupled with the flow cytometer CytoFLEX (Beckman Coulter, Brea, USA). The OC-300 performs the automatic sampling, dilution, fixation and 4',6-diamidino-2-phenylindole (DAPI) staining of a bacterial sample before sending it to the CytoFLEX for measurement. The resulting data can be analysed in an automated manner using the flowEMMi v2 tool, allowing it to be fed into available bioinformatics tools.

Impact

- Online automated flow cytometry can rapidly provide individual cell data that reflect bacterial community status. This enables highly time-resolved monitoring of a population's dynamics which is essential for controlling biotechnological processes that use bacterial communities. This is of particular relevance for the intended **transition to a circular bioeconomy, which is based on the use of microbial consortia for the revalorisation of waste.**
- The online automated flow cytometry procedure is capable of providing the same high-resolution fingerprints, i.e. profiles of the bacterial community structure, as a more tedious manual procedure and enables their automated analysis and feeding into available bioinformatics tools. This makes the **data available in a very short time, eliminates human error and allows for bioreactors to be controlled online.**

Source

López-Gálvez, J., Schiessl, K., Besmer, M. D., Bruckmann, C., Harms, H., & Müller, S. (2023). Development of an Automated Online Flow Cytometry Method to Quantify Cell Density and Fingerprint Bacterial Communities. *Cells,* 12(12), 1559. https://doi.org/10.3390/cells12121559

A GENERAL DEEP HYBRID MODEL FOR BIOREACTOR SYSTEMS: COMBINING FIRST PRINCIPLES WITH DEEP NEURAL NETWORKS

Key message

These findings hold immense value for bioprocess engineers and researchers invested in optimizing bioreactor systems as the proposed general deep hybrid modeling technic can unlock a deeper understanding and control of biological processes.

Background

Mathematical models, represented by equations, simulate biological reactors and play a crucial role in optimizing processes that utilize microorganisms to produce valuable products. Traditionally, scientists have relied on a combination of fundamental physical laws (First Principles) and basic artificial intelligence (shallow neural networks) to model these systems. However, recent breakthroughs in deep learning (more advanced neural networks) present an exciting opportunity to create more powerful and accurate models.

Objective

This study aimed to enhance bioreactor modeling by seamlessly integrating deep neural networks with First Principles. The result is an improved model performance, including better predictions and enhanced generalization.

Source

Pinto, J., Mestre, M., Ramos, J., Costa, R. S., Striedner, G., & Oliveira, R. (2022). A general deep hybrid model for bioreactor systems: Combining first principles with deep neural networks. *Computers & Chemical Engineering*, 165, 107952. Available from: https://doi.org/10.1016/j.compchemeng.2022.107952

Findings

Deep Hybrid Models: A novel paradigm combining the complexity of deep neural networks with the rigor of First Principles equations was introduced.

Advanced and efficient Training Techniques: Various deep learning methods were compared resulting in 43.4% faster deep hybrid model training compared to traditional shallow models.

Real-World Applications: The methods were put to the test using both synthetic data and a real bioprocess data from a pilot 50L bioreactor.

Accuracy Boost: Deep hybrid models consistently outperformed their shallow counterparts, boasting an impressive 18.4% increase in prediction accuracy.

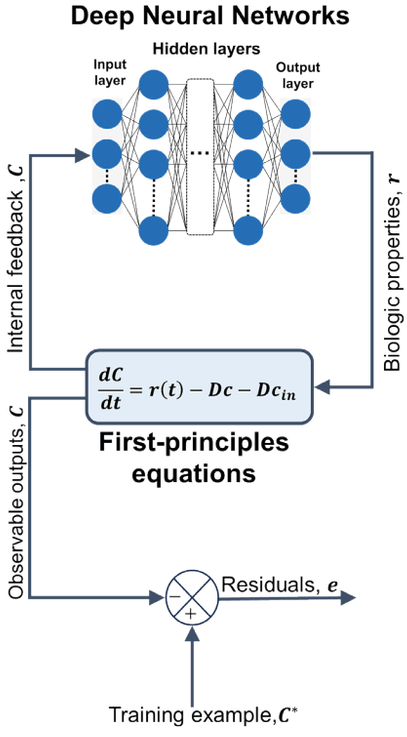
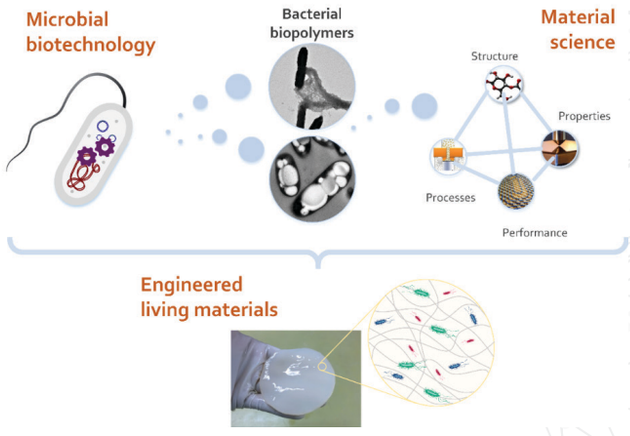


Figure. Deep hybrid model structure for bioreactor systems.

PROMICON

WHEN MICROBIAL BIOTECHNOLOGY MEETS MATERIAL ENGINEERING



Key message

This study highlights the enormous structural diversity of biopolymers, their production processes and how they can be modified, both biologically and chemically. It also explores the wide range of potential applications of these macromolecules in our daily lives, focusing on the rapidly growing field of engineered living materials (ELMs): bringing bacterial biopolymers to life.

Background

Bacterial biopolymers such as bacterial cellulose (BC), alginate or polyhydroxyalkanotes (PHAs) have aroused the interest of researchers in many fields, for instance, biomedicine and packaging, due to their being biodegradable, biocompatible and renewable. By employing microbial biotechnology strategies and materials science, the physicochemical, thermodynamic and mechanical properties of these biopolymers can be fine-tuned, giving rise to biopolymers with a wide range of non-native attributes.

Take-home messages

- This study emphasises the extraordinary diversity of bacterial biopolymers, which can be greatly expanded through the synergistic combination of microbial biotechnology, synthetic biology, metabolic engineering, and materials science.
- Bacteria are capable of naturally synthesising a wide range of biopolymers like polyamides, polysaccharides, polyesters and polyphosphates.
- Nonetheless, diversification thereof through metabolic engineering and chemical modification of their side chains or via crosslinking with other biopolymers/molecules, provides even more potential functionalities.
- The potential for bacterial biopolymers to be used as scaffolds for living organisms opens the door to creating ELMs capable of self-repair and responding to stimuli.

Source

Hernández-Arriaga, A.M., Campano, C., Rivero-Buceta, V., Prieto, M.A. (2022). When microbial biotechnology meets material engineering. *Microb Biotechnol*, 15(1): 149–163. https://doi.org/10.1111/1751-7915.13975

Results

- Bacteria naturally produce various polymers as part of their inherent physiology in the form of storage molecules, protective capsular layers surrounding cells, or as an extracellular matrix. Apart from natural proteins and nucleic acids, bacteria can produce polyamides, polyesters, polyphosphates (polyP), polysaccharides and extracellular recombinant proteins. These have applications in polymer biotechnology field. By manipulating the molecular weight, charge, monomer composition and specific 3D structure, a range of thermo-chemical and mechanical properties can be achieved.
- Chemical modification techniques such as blending, grafting/crosslinking and curing, further expand the potential uses of these biopolymers by introducing functional groups of interest, which would make them suitable for high value-added applications.
- The emergence of new technologies, such as synthetic biology, enables the creation of next-generation-advanced materials presenting smart functional properties, for example the ability to sense and respond to stimuli as well as the capacity for self-repair. These advances have led to the development of biohybrid materials, where synthetic components are combined with living organisms.
- Two different subfields have recently garnered particular attention: hybrid living materials (HLMs) and engineered living materials (ELMs). HLMs involve encapsulation or bioprinting, while ELMs are built from scratch using microbial biotechnology tools. Early studies showed the strong potential of alginate and PHAs as HLMs, whilst BC constituted the most currently promising material for the creation of ELMs.

BIOPLASTIC PRODUCTION BY HARNESSING CYANOBACTERIA-RICH MICROBIOMES FOR LONG-TERM SYNTHESIS

Key message

For the first time, this study demonstrates that it is possible to produce PHB using a photosynthetic microbial community over the long term in a non-sterile environment. This breakthrough brings us closer to developing more sustainable methods for producing biodegradable plastics, while also providing new insights into the biological processes involved in PHB production.

Objective

This research explores a new method of producing PHB by shifting away from traditional approaches that rely on sterile and heterotrophic cultures. Instead, it studied the potential of using photosynthetic microorganisms dominated by cyanobacteria *Synechocystis* sp. and *Synechococcus* sp., cultivated in a 3 L photobioreactor. These microorganisms use CO2 and sunlight to grow and could offer a more sustainable way to produce PHB.

Background

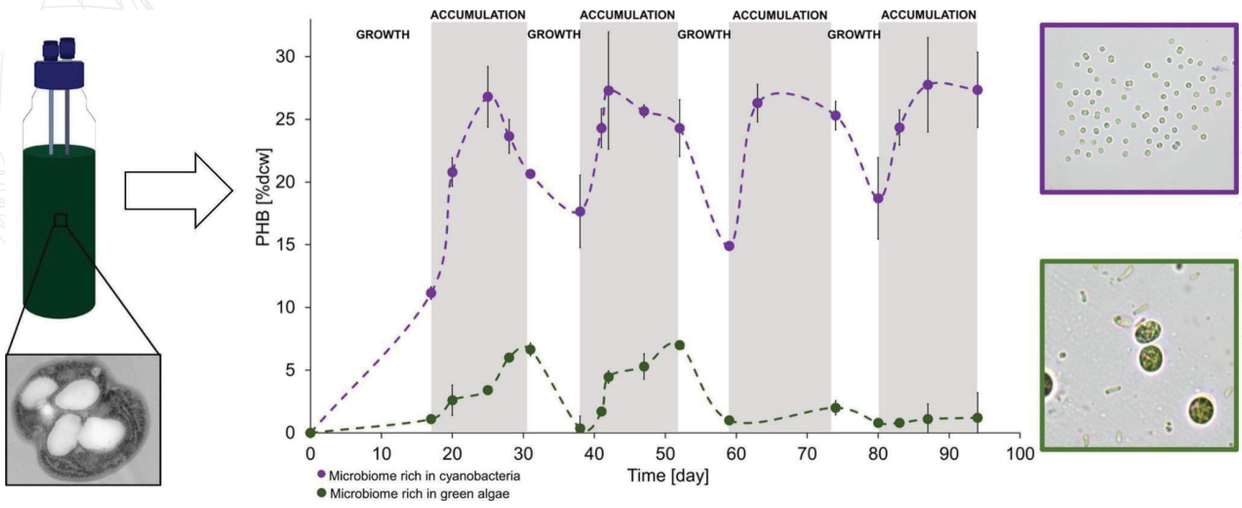
Polyhydroxybutyrate (PHB) is a type of biodegradable plastic that could become an environmentally friendly alternative to conventional plastics. However, there are still significant challenges to producing PHB on a large scale in an efficient and cost-effective way.

Results

- Over a period of 108 days, the microbiome was able to produce PHB, reaching up to 28% of the dry weight of the cells.
- Nile Blue staining and Transmission Electron Microscopy confirmed the presence of PHB granules inside the cyanobacteria.
- The overexpression of the enzyme PHA synthase correlated directly with the increased PHB production.
- It was confirmed that the biopolymer produced was specifically poly-3-hydroxybutyrate.

Source

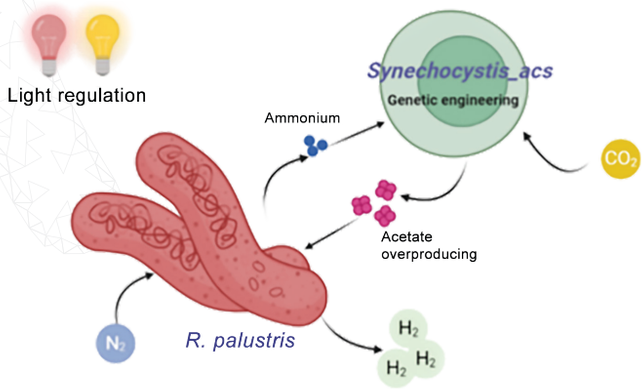
Altamira-Algarra, B., Lage, A., Meléndez, A. L., Arnau, M., Gonzalez-Flo, E., & García, J. (2024). Bioplastic production by harnessing cyanobacteria-rich microbiomes for long-term synthesis. *Science of The Total Environment,* 954, 176136. https://doi.org/10.1016/j.scitotenv.2024.176136



ENGINEERING A PHOTOAUTOTROPHIC MICROBIAL COCULTURE TOWARD ENHANCED BIOHYDROGEN PRODUCTION

Key message

The application of synthetic phototrophic microbial consortia holds promise for sustainable bioenergy production. Nevertheless, strategies for the efficient construction and regulation of such consortia remain challenging. Applying tools of genetic engineering and light regulation, this study successfully constructed a synthetic community of phototrophs, enabling the production of biohydrogen and fatty acids through nitrogen and carbon dioxide fixation. This approach demonstrates a promising strategy for sustainable bioenergy production through the integration of genetic engineering and light regulation.



Background

Phototrophic microbial communities are commonly found in light-exposed environments. Such light-driven consortia contribute substantially to the global primary production of organic compounds by fixing carbon dioxide and/or nitrogen gas. With humankind facing ever-growing energy demands and environmental problems, such synthetic phototrophic consortia may provide a promising alternative to current energy generation methods. These consortia can efficiently convert CO_2 and N_2 gases together with water and solar energy into bioenergy products.

However, when attempting to create a synthetic microbial consortium outside the specific environmental conditions of its natural habitat, one strain may outcompete the others and dominate the community. This imbalance can compromise the stability of the consortium. Therefore, developing effective strategies to maintain strain equilibrium and regulate the overall functionality of the consortium remains a significant challenge.

Objective

This study set out to construct and successfully regulate a phototrophic community, enabling H_2 and fatty acid production through carbon and nitrogen fixation. It cocultivated a community of phototrophs using *Rhodopseudomonas palustris* (*R. palustris*) with either the wild type of *Synechocystis sp.* PCC 6803, or an engineered strain, *Synechocystis_acs* (an acetate overproducing strain). Various light regulation strategies, including constant illumination, circadian light-dark illumination, and circadian light-infrared illumination, were employed. These strategies facilitated trophic dependence through carbon and nitrogen assimilation and allowed for the regulation of coculture growth. The coculture enabled biohydrogen production in a light-based system feeding on CO_2 and N_2, highlighting the potential of controlling a phototrophic community.

Results

- Elemental balance confirmed carbon capture and nitrogen fixation into the consortium.
- The strategy of circadian illumination effectively limited oxygen levels in the system, ensuring the activity of the nitrogenase in *R. palustris*, despite oxygenic photosynthesis happening in *Synechocystis*.
- When infrared light was introduced into the circadian illumination, the production of H_2 (9.70 μmol mg−1) and fatty acids (especially C16 and C18) was significantly enhanced.
- Proteomic analysis indicated acetate exchange and light-dependent regulation of metabolic activities.
- Infrared illumination significantly stimulated the expression of proteins coding for nitrogen fixation, carbohydrate metabolism, and transporters in *R. palustris*, while constant white light led to the most upregulation of photosynthesis-related proteins in *Synechocystis_acs*.

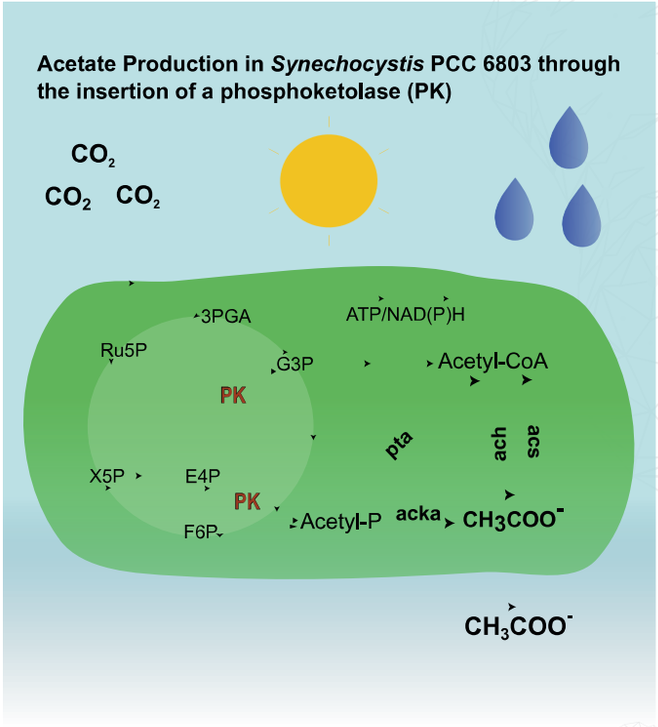
Source

Pan, M., Colpo, R. A., Roussou, S., Ding, C., Lindblad, P., & Krömer, J. O. (2025). Engineering a Photoautotrophic Microbial Coculture toward Enhanced Biohydrogen Production. *Environmental Science & Technology*, 59(1), 337–348. https://doi.org/10.1021/acs.est.4c08629

EXPLORING AND INCREASED ACETATE BIOSYNTHESIS IN *SYNECHOCYSTIS* PCC 6803 THROUGH INSERTION OF A HETEROLOGOUS PHOSPHOKETOLASE AND OVEREXPRESSING PHOSPHOTRANSACETYLASE

Key message

The study successfully enhanced acetate production in *Synechocystis* PCC 6803 by introducing a heterologous phosphoketolase (PKPa) and overexpressing phosphotransacetylase (Pta), leading to a significant increase in acetate secretion (up to 2.3 g/L) which is 80 folds more than the wild type. Insertion of the phosphoketolase showed 40 times increase while metabolite analysis also showed enhanced Calvin-Benson-Bassham cycle.



Background

Acetate is an important industrial compound produced biologically by various microorganisms, including cyanobacteria. *Synechocystis* PCC 6803 can produce acetate under specific conditions, but only in low amounts. By modifying metabolic pathways, particularly dragging more carbon to acetyl-phosphate (precursor of the acetate), it is possible to improve acetate synthesis and secretion under photosynthetic conditions.

Objective

To enhance acetate production in *Synechocystis* PCC 6803 by inserting a heterologous phosphoketolase (PKPa) and exploring the key enzymes in the acetate production pathway, including knocking out or overexpress phosphotransacetylase (Pta), acetate kinase (AckA), and acetyl-CoA hydrolase (Ach).

Source

Roussou, S., Pan, M., Krömer, J. O., & Lindblad, P. (2025). Exploring and increased acetate biosynthesis in *Synechocystis* PCC 6803 through insertion of a heterologous phosphoketolase and overexpressing phosphotransacetylase. *Metabolic Engineering*, 88, 250–260. https://doi.org/10.1016/j.ymben.2025.01.008

Results

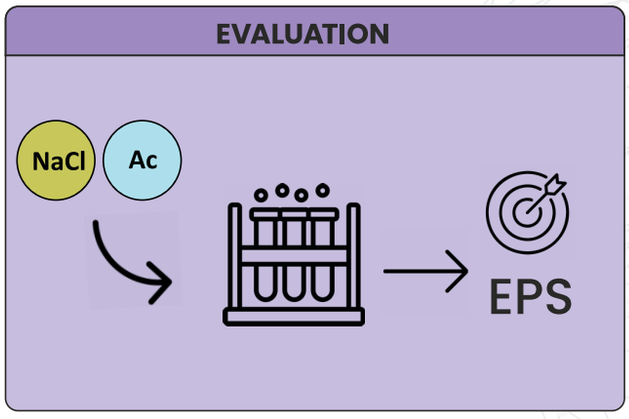
- **Insertion of PKPa** led to a **40-fold** increase in extracellular acetate compared to wild-type strains.
- **Overexpression of Pta** (in combination with PKPa) further increased acetate production to **80 times the wild-type levels**, reaching **2.3 g/L after 14 days**.
- **Metabolomic analysis** showed increased levels of acetyl-phosphate, fructose-1,6-bisphosphate, and erythrose-4-phosphate, indicating enhanced carbon flux.
- **Knocking out key acetate pathway enzymes (Ach, Pta, AckA)** affected acetate levels differently, with Pta being the most influential in acetate production.
- **Acetate secretion appeared efficient**, suggesting an active transport mechanism rather than passive diffusion.

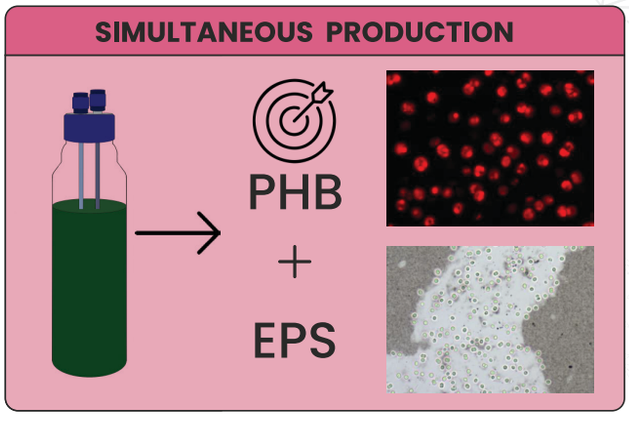
PROMICON

EXPLORING SIMULTANEOUS PRODUCTION OF POLY(3-HYDROXYBUTYRATE) AND EXOPOLYSACCHARIDES IN CYANOBACTERIA-RICH MICROBIOMES

Main message

- Cyanobacterial microbiomes are capable of consistent EPS production, even under varying environmental conditions.
- While acetate and salt can influence specific microbiomes, their overall impact on EPS synthesis is limited.
- The presence of uronic acid in EPS can be beneficial for biomass separation.*
- The visualization techniques are very important for the analysis of the polymers.





Objective

The aim of this study was to explore the viability of the dual production of poly(3-hydroxybutyrate) (PHB) and exopolysaccharides (EPS) by seven microbiomes rich in cyanobacteria.

Highlights

- Field microbiomes rich in cyanobacteria were evaluated for EPS production.
- Acetate and salt additions minimally affected EPS synthesis rates and composition.
- Glucose dominated as the main monosaccharide in microbiomes' polysaccharides.
- Staining revealed PHB granules in cyanobacteria and EPS around cells.
- Simultaneous 205 mg L^{-1} EPS and 87 mg L^{-1} PHB production was achieved in a 3 L photobioreactor.

Source

Altamira-Algarra, B., García, J., Torres, C. A. V., Reis, M. A. M., & Flo, E. G. (2025). Exploring simultaneous production of poly(3-hydroxybutyrate) and exopolysaccharides in cyanobacteria-rich microbiomes. *New Biotechnology*, 87, 82–92. https://doi.org/10.1016/j.nbt.2025.02.008

CYANOBACTERIA MICROBIOMES FOR BIOPLASTIC PRODUCTION: CRITICAL REVIEW OF KEY FACTORS AND CHALLENGES IN SCALING FROM LABORATORY TO INDUSTRY SET-UPS

Main message

Cyanobacteria-rich microbiomes present a promising avenue for sustainable and high-yield PHB production under non-sterile conditions, offering new opportunities for the bioplastics industry. Achieving optimal PHB yields necessitates careful control of microbial community composition and cultivation parameters. Advanced analytical techniques enable real-time monitoring and analysis. While scaling up remains a challenge, a two-stage cultivation strategy, focusing on biomass growth followed by PHB synthesis, holds significant potential for achieving large-scale, cost-effective PHB production using these microbiomes in open systems.

Background

Current industrial-scale polyhydroxybutyrate (PHB) production primarily uses heterotrophic bacteria (e.g., *Cupriavidus necator*, *Halomonas* sp., recombinant *Escherichia coli*) cultivated on refined feedstocks like glucose or sucrose, leading to high production costs. To explore more sustainable and cost-effective alternatives, **photoautotrophic cyanobacteria, which use CO2 and sunlight rather than organic carbon, are being explored**. While cyanobacteria are promising platforms for various high-value products, their application in PHB production remains limited compared to heterotrophic systems, representing less than 5% of related scientific publications. Furthermore, the use of mixed microbial consortia (microbiomes) is being explored to enhance process robustness and flexibility, potentially enabling non-sterile cultivation and the utilisation of low-cost waste streams. Although research on cyanobacterial microbiomes for PHB production is scarce, the combined benefits of cyanobacteria and microbiome approaches present a significant area for future development.

Objective

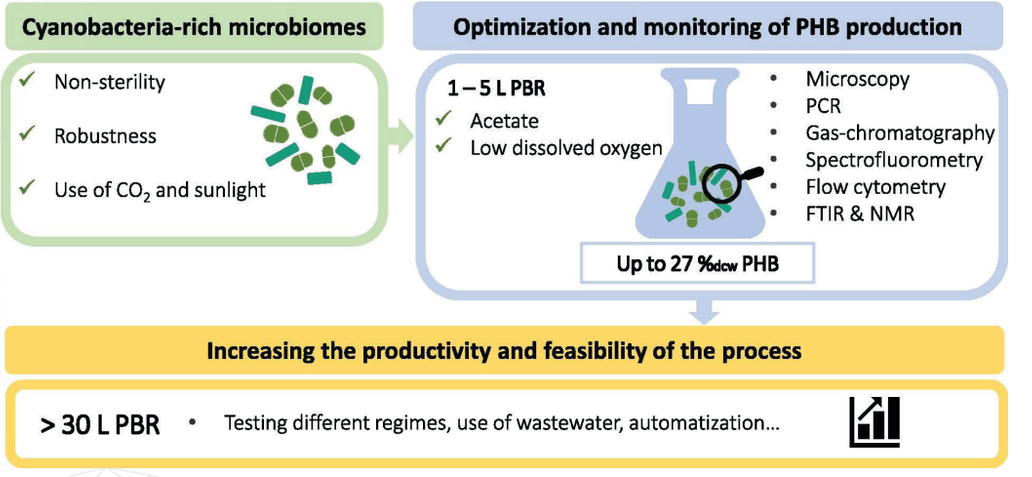
Managing microbiome population dynamics in non-sterile environments requires effective monitoring and control. Overcoming these challenges involves integrating molecular biology techniques with quantitative and qualitative PHB analysis. While cyanobacteria microbiomes show promise for PHB production, optimising strategies is essential to address non-sterile conditions and scalability. The transition from lab-scale to industrial-scale PHB production remains complex, requiring carefully integrated approaches.

Highlights

- Cyanobacteria microbiomes offer a method for PHA production in non-sterile setups.
- Cyanobacteria microbiomes show great long-term productivity.
- Sustained production has allowed to achieve 27% dcw PHB yield over time.
- Scaling up PHB production poses challenges but promising strategies are explored.
- Microbiome cultures reduce scaling risks versus current PHB production methods.

Source

Altamira-Algarra, B., Garcia, J., Gonzalez-Flo, E. (2025). Cyanobacteria microbiomes for bioplastic production: Critical review of key factors and challenges in scaling from laboratory to industry set-ups. *Bioresource Technology*, Volume 422, Article 132231. https://doi.org/10.1016/j.biortech.2025.132231



Policy briefs

Strengthening the 2018 EU Bioeconomy Strategy through Microbiome Analysis and Synthetic Microbial Consortia Technologies

Policy brief 1

INTRODUCTION

The 2018 EU Bioeconomy Strategy aims to create a sustainable, circular, and low-emissions economy, based on the use of renewable biological resources. The strategy focuses on the development of new bio-based products and markets, the promotion of sustainable and efficient use of resources, and the support of research, innovation, and skills development in the bioeconomy sector. The main objectives of the strategy are to:

- Accelerate the deployment of bio-based products and services to reduce Europe's dependence on fossil fuels and promote the transition to a circular economy;
- Optimise the use of renewable biological resources to ensure food security, while also protecting the environment and biodiversity;
- Promote sustainable and efficient use of natural resources, reduce waste, and minimize greenhouse gas emissions;
- Develop innovative, sustainable, and competitive bio-based industries and value chains that create jobs and support economic growth, particularly in rural areas;
- Support research, innovation, and skills development in the bioeconomy sector to drive technological advancements, enhance the competitiveness of the EU's bio-based industries, and address societal challenges such as climate change, food security, and public health.

The EU Horizon 2020 PROMICON project is a pioneering initiative that directly addresses the objectives of the 2018 EU Bioeconomy Strategy. By focusing on the production of biopolymers, energy carriers, feedstocks, and antimicrobial molecules from natural microbiomes, the project is promoting the sustainable production and use of renewable biological resources while also supporting research and innovation in the bioeconomy sector.

EVIDENCE AND ANALYSIS

The PROMICON project aims to develop technologies for the study and understanding of microbial consortia, with a focus on biotechnology applications. The project is developing methods for the assembly of minimal functional consortia for production, while still maintaining the self-stabilising characteristics of natural microbiomes. These synthetic microbial consortia will consist of bacterial farmers, producers, and stabilisers, and will be optimised through systems metabolic engineering and synthetic biology to provide optimal production of target metabolites.

The PROMICON project, through two of its work packages, will generate policy-relevant findings that are important for the EU's 2018 bioeconomy strategy. Work package 1 is focused on developing analysis and modelling approaches for microbiomes, including a standardised platform for obtaining data sets, which will enable the optimisation of microbiomes for industrial applications. The development of these approaches will facilitate the identification of key species and processes that can be leveraged for the production of biopolymers, energy carriers, drop-in feedstocks, and antimicrobial molecules.

Work package 3, on the other hand, aims to optimise the minimal functional metabolic modules in a target bioprocess and reassemble them in the context of a synthetic microbial consortium for optimal production of target metabolites. This approach will allow for the selection or optimisation of bacterial farmers, producers, and stabilisers through systems metabolic engineering and synthetic biology. The findings of these two work packages are highly relevant to the EU's Bioeconomy Strategy, which seeks to transition to a sustainable, circular, and low-carbon economy. By harnessing the potential of microbiomes for the production of valuable products, the PROMICON project can contribute to the EU's goal of reducing its dependence on fossil fuels and promoting the efficient use of resources. Furthermore, the project's focus on synthetic biology and systems metabolic engineering aligns with the EU's objective of fostering innovation and developing new technologies for sustainable production. Overall, the PROMICON project's work packages 1 and 3 provide important insights into how microbiomes can be optimised for industrial applications, which can contribute to the achievement of the EU's bioeconomy strategy goals.

POLICY IMPLICATIONS AND RECOMMENDATIONS

In an effort to strengthen and update the 2018 EU Bioeconomy Strategy, PROMICON proposes the following policy recommendations:

- Supporting research and development efforts focused on learning from nature and developing enabling technologies for microbiome analysis and modeling, particularly targeting academic institutions, research organisations, and scientific communities involved in microbiome research.
- Encouraging companies from the agriculture, biotechnology, or pharmaceuticals sector to invest in the development of synthetic microbial consortia for the production of target metabolites, as outlined in Work Package 3 of the EU Bioeconomy Strategy.
- Ministries, policymakers, and regulatory bodies should promote regulatory frameworks that support the use of synthetic biology in industrial applications, fostering interdisciplinary collaboration and knowledge-sharing among researchers, policymakers, and industry stakeholders. The target groups include government bodies responsible for bioeconomy policies, regulatory agencies, research institutions, and industry associations.
- Encouraging the adoption of circular economy principles in the bioeconomy, with a focus on reducing waste and promoting resource efficiency. This recommendation specifically targets industries and businesses operating in the bioeconomy sector, waste management organisations, and relevant government bodies responsible for promoting circular economy initiatives.

SUSTAINABILITY AND LEGACY

Deliverable D1.9, explores the natural microbial community and highlights the value of learning from nature. On the other hand, D3.3 and D3.6, focuses on creating a suitable *Pseudomonas* strain for labor purposes, specifically by modifying its sucrose metabolism and eliminating negative traits. D3.6 involves developing an optimised set of *P. taiwanensis* VLB 120 balancer strains with increased c-di-GMP levels. These two deliverables complement D1.9 by providing comprehensive information on genetically modified strains that can produce sucrose, PHA, and acetate. There is a strong relationship between these latter two deliverables and D1.1, D1.4, and D1.8 because the results in each report are interconnected. This connection allows PROMICON experts to conduct a coherent assessment of performance and characterise potential effects or changes in the production processes based on the information presented in D3.3 and D3.6.

Supporting the transition to a circular bioeconomy with improved online monitoring of microbial communities

Policy brief 2

INTRODUCTION

Europe has been facing a complex web of challenges due to the growing demand for biological resources, essential to secure food supplies, biomaterials, biofuels and bio-based products. However, the capacity of many ecosystems to provide such goods is being jeopardised by unsustainable exploitation and climate change leading to their degradation.

In this context, the European Union has turned to the sustainable bioeconomy as a new way of producing and consuming resources while respecting our planetary boundaries and moving away from a linear economy based on extensive use of fossil energy and mineral resources [1]. The bioeconomy can act as a catalyst for the economic, social, and environmental transformations required by the European Green Deal by reducing dependence on fossil fuels, delivering on Europe's economic prosperity, ensuring a fair and just transition, and enhancing the protection of the environment and ecosystems.

To maximise the contribution of the bioeconomy to major European policy priorities, the European Commission adopted a Bioeconomy Strategy in 2012 and updated it in 2018 [2]. The strategy has five objectives:

1. Ensuring food and nutrition security;
2. Managing natural resources sustainably;
3. Reducing dependence on non-renewable, unsustainable resources;
4. Limiting and adapting to climate change;
5. Strengthening European competitiveness and creating jobs.

The Commission will review the EU Bioeconomy Strategy by the end of 2025, considering current societal, demographic, and environmental challenges, while strengthening the bioeconomy's industrial dimension and its links to biotechnology and biomanufacturing to bolster the EU economy [3].

To support the objectives of the Bioeconomy Strategy, the European Commission has funded numerous bioeconomy projects. Specifically, the 'Food security, sustainable agriculture and forestry, marine, maritime and inland water research, and the bioeconomy' work program aimed to ensure a sufficient supply of safe, high-quality food and bio-based products [4]. This included projects aimed at understanding, monitoring and exploiting microbial communities in the industrial environment, such as PROMICON (No. 101000733).

EVIDENCE AND ANALYSIS

Biotechnology – combined with the application of our knowledge of complex microbial communities from various environments and ecosystems (microbiomes) – can contribute to the fight against climate change [3]. To expand the applications of microbial communities in biotechnological processes, thus supporting the transition to a circular bioeconomy, participants of the international EU-funded project PROMICON propose an automated online monitoring system. The full extent of the work and results described below are available in López-Gálvez, J. *et al*. (2022) and López-Gálvez, J. *et al*. (2023).

A circular bioeconomy is one of the main components for sustainable development required in the near future. This type of economy is largely based on the use of microbial consortia for the biotransformation of waste into valuable products, most notably biofuels [7, 8]. However, the use of microbial communities in biotechnological processes is currently limited due to the complexity of controlling their composition and functionality. Obtaining frequent and reliable information on the composition and active members of a bacterial community is, therefore, an essential first step for gaining proper control of it.

One of the key technologies for microbial communities monitoring is flow cytometry. Conventional offline flow cytometry has successfully been applied as a measuring tool in various settings, including in wastewater [9] or in anaerobic digesters [10], as well as for control of *E. coli* and *S. cerevisiae* cultures in bioreactors [11] and for the profiling of different *Lactobacillus strains* [12]. Beyond biotechnology, the offline flow cytometry of microbial communities' states has a wide range of applications, e.g., in drinking water [13, 14] or for electricity generation [15, 16].

Fully automated online flow cytometry is already applied to drinking water systems for measuring cell concentrations [17, 18] or live/dead cell proportions [19], as well as being used to measure pure cultures, for instance, lipid accumulation and the cell growth of yeast [20]. However, an online analysis procedure of complex microbial communities that can distinguish a large number of subcommunities in microbial bioprocesses with typically high cell densities is not yet feasible.

To overcome these limitations, López-Gálvez, J. *et al*. (2023) suggest using an automated monitoring system. The system includes hardware, software and an automated process to keep track of the absolute cell abundance and community composition with a high temporal resolution in dense samples that are typical for biotechnology.

POLICY IMPLICATIONS

The various bioeconomy sectors rely on ecosystem services and resources to produce food, feed, bio-based products, energy and services. The transition to a sustainable and circular bioeconomy is stipulated by several EU policies and initiatives, including the Bioeconomy Strategy and the Circular Economy Action Plan.

In the context of the bioeconomy, microbial communities have inspired a rapidly growing interest in industrial processes due to their appealing features such as stability, functional robustness and the ability to perform complex tasks. However, such consortia are still facing challenges such as low efficiencies and instability. In this sense, real-time monitoring could contribute significantly to the decision-making of regulatory strategies. To provide a rapid and reliable means for the monitoring of bacterial communities, PROMICON researchers propose a novel online automated flow cytometry procedure. Online flow cytometry has great potential for different biotechnological applications, especially for knowing the growth and response of each individual in a complex consortium.

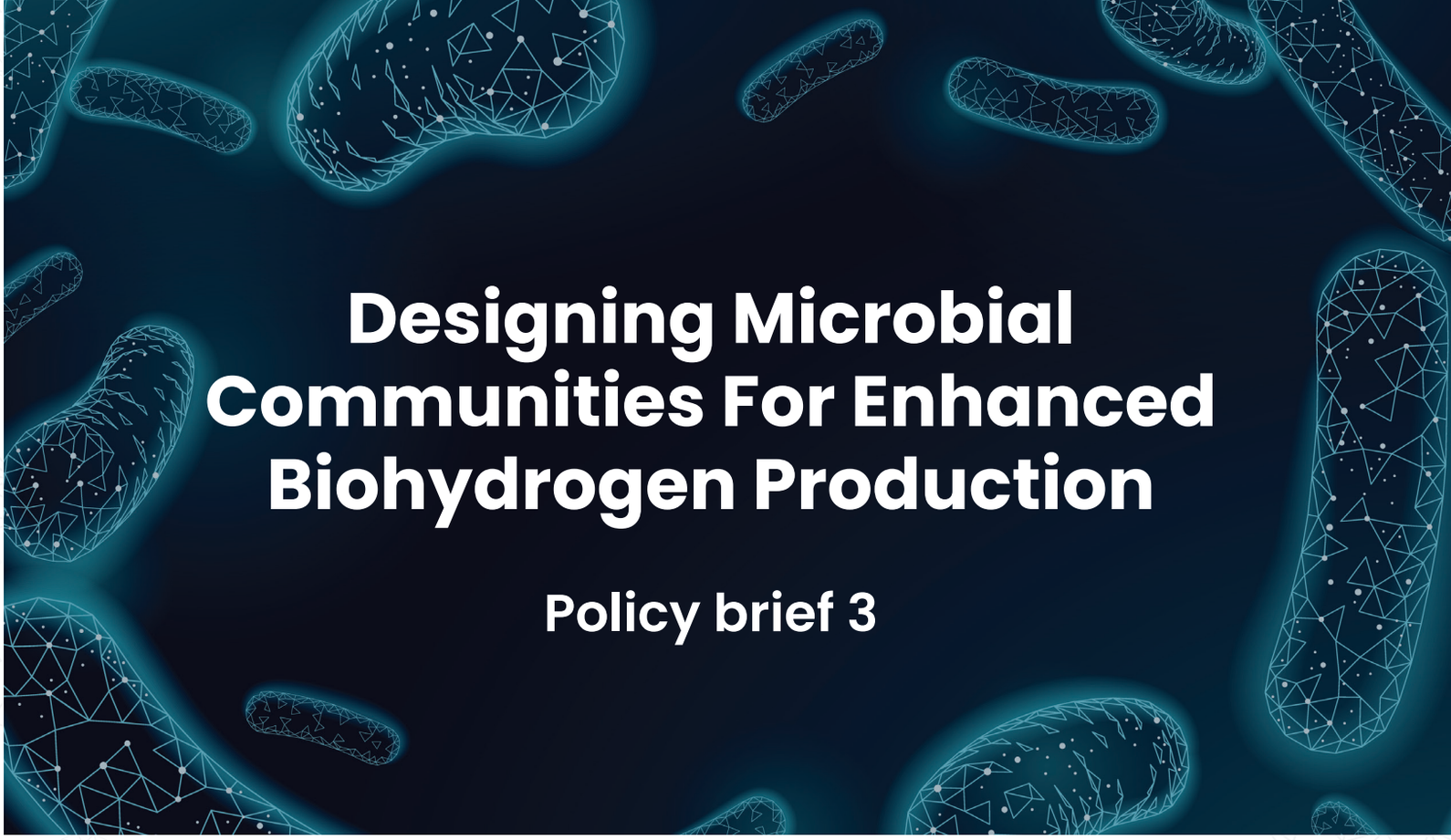
To support the transition to a circular bioeconomy and improve the use of microbial communities in biotechnological processes, PROMICON (López-Gálvez, J. *et al*., 2023) suggests expanding the use of this novel online automated flow cytometry procedure which offers the following benefits:

- It can rapidly provide individual cell data that reflect the status of a bacterial community. A fast acquisition of information allows for timely monitoring and can be used as a basis for properly controlling the biotechnological processes that use bacterial communities. This is of particular relevance for the intended transition to a circular bioeconomy, which is based on the use of microbial consortia for the revalorization of waste.
- The system includes an automated workflow with a high frequency of sampling (every 45 min) without the need for an operator, thereby eliminating human error and making the process less time-consuming. The delay between manual sampling and obtaining biomass information is otherwise substantial and hinders meaningful and fast decision-making.
- The resulting data can be analysed in an automated manner with the flowEMMi v2 tool, allowing it to be fed into available bioinformatics tools. This makes the data available in a very short time, allowing for the bioreactors to be controlled online.

SUSTAINABILITY AND LEGACY

The methodology, results and implications of the proposed online automated flow cytometry procedure are documented in PROMICON's Deliverable D1.4 (López-Gálvez, J. *et al*., 2022) and the subsequent publication by López-Gálvez, J. *et al*. (2023) which is openly available at https://doi.org/10.3390/cells12121559. Furthermore, its supporting information can be downloaded at https://www.mdpi.com/article/10.3390/cells12121559/s1.

Scan the QR code for the reference list



Designing Microbial Communities For Enhanced Biohydrogen Production

Policy brief 3

INTRODUCTION

The widespread use of petrol-based plastics has led to an environmental problem, as these materials are prone to abandonment, breaking down into microplastics and nanoplastics that harm living organisms. While biodegradable plastics are seen as a solution, their global production still remains modest at 1.3 million tons in 2022 (vs. 400 million tons of petrol-based plastics)[1]. Moreover, many such plastics fail to biodegrade efficiently under all environmental conditions (marine, soil, rivers, etc.)[2].

Polyhydroxyalkanoates (PHA) are a type of bioplastics naturally produced by microorganisms. They are a promising alternative because they degrade completely in soil, water, and marine environments. However, their industrial production is still limited and needs further research and investment to scale up.

Commercially produced PHA is nowadays highly energy-intensive and relies heavily on organic raw materials and clean water, which conflicts with the EU's goals for a circular, sustainable economy. The current production process is far away from the zero emissions neutral carbon strategy. The EU Horizon 2020 **PROMICON project** has developed an innovative method that uses photosynthetic microorganisms (cyanobacteria) to produce PHA efficiently. This process uses sunlight, absorbs CO_2, and requires minimal organic resources, aligning perfectly with EU bioeconomy goals.

EVIDENCE AND ANALYSIS

The PROMICON project has made significant progress in green PHA production:

- Demonstrated **continuous PHA production** over 100 days using cyanobacteria, overcoming the short timescales of previous studies[3].
- Discovered that PHA production occurs in two phases (growth and accumulation), opening the door to **scaling up the process**[4].
- Developed methods to **convert CO_2 directly into bioplastics**, reducing the need for plant-based sugars and fertilisers used in conventional production.

This process eliminates the need for aeration (a major energy cost), captures 2 kg of CO_2 per kg of biomass, and creates a truly biodegradable plastic alternative that leaves no microplastic residues.

POLICY IMPLICATIONS AND RECOMMENDATIONS

To support the development of this groundbreaking technology, we recommend the following actions:

- **Increase funding** for research on cyanobacteria-based PHA production to scale it up for industrial use.
- **Incentivise industry** adoption by creating certifications and labels for PHA products.
- **Encourage applications** of PHA in sectors prone to plastic waste, such as agriculture.
- **Raise awareness** through educational campaigns about PHA's environmental benefits.
- **Establish global standards** for biodegraadability testing by fostering international collaboration.

SUSTAINABILITY AND LEGACY

Cyanobacteria-based PHA production aligns with key sustainability goals:

- **Reduces plastic pollution** by producing fully biodegradable plastics.
- **Cuts CO_2 emission**s by using photosynthesis to convert CO_2 into useful materials.
- **Promotes resource efficiency** by reducing dependence on fossil fuels and agricultural inputs.

This innovation supports a transition to sustainable bioplastics, reduces environmental damage, and creates green jobs, positioning the EU as a leader in the bioeconomy.

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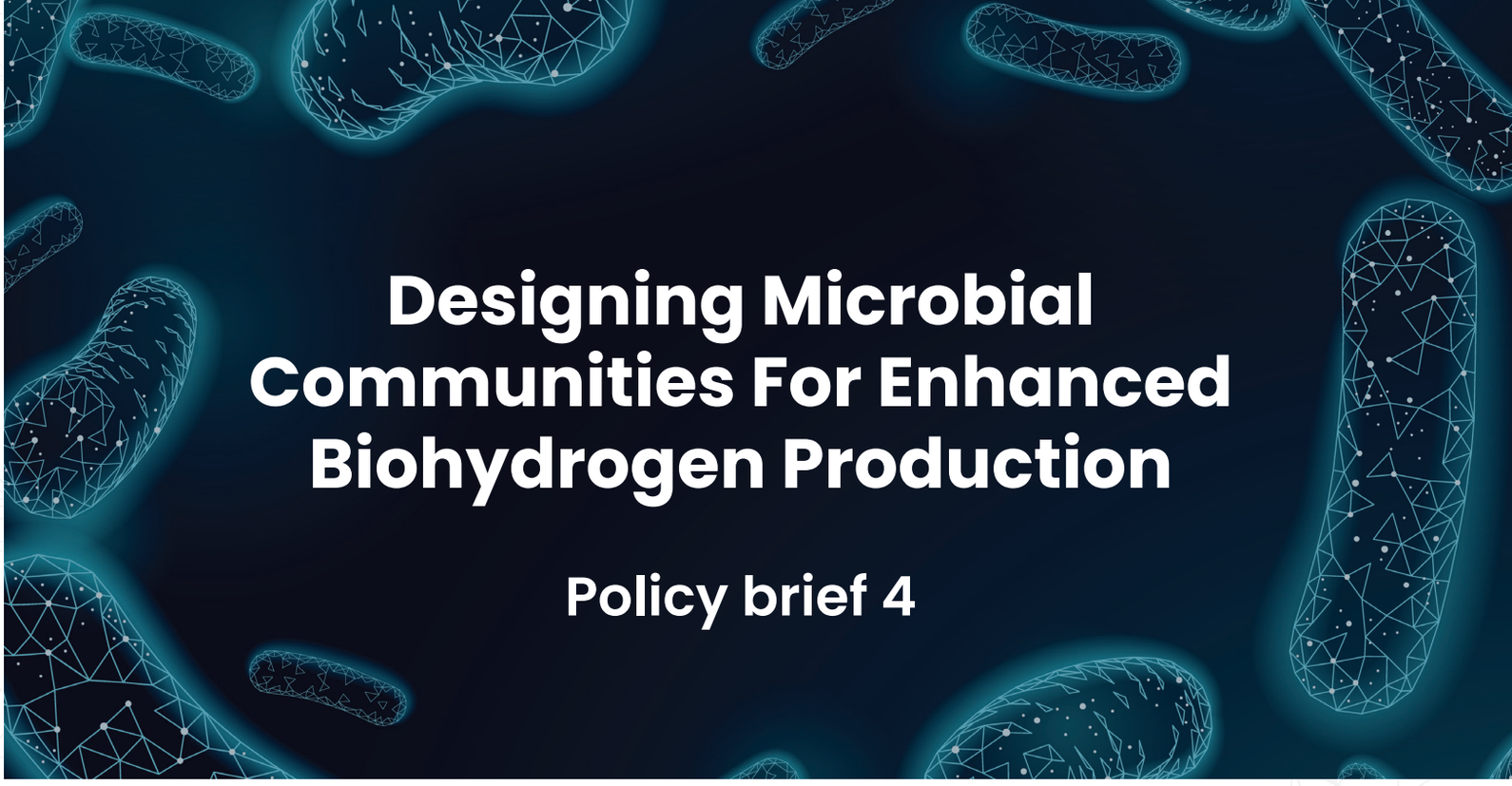

Designing Microbial Communities For Enhanced Biohydrogen Production

Policy brief 4

INTRODUCTION

Phototrophic microbial communities – groups of tiny organisms whose energy for growth comes from light – play a significant role in global primary production by absorbing carbon dioxide and nitrogen gas. With the growing challenges of energy demands and environmental concerns, researchers are exploring scientifically designed (synthetic) phototrophic communities as a promising alternative to traditional energy generation methods. These consortia can efficiently convert CO_2 and N_2 gases, along with water and solar energy, into bioenergy products, offering a potential solution to today's energy and sustainability problems.

In this context, the development of synthetic phototrophic communities has attracted increased attention due to their ability to divide tasks among different species, allowing them to function more efficiently and remain stable. However, challenges remain, particularly in maintaining balance among strains and ensuring stable performance in environments that do not replicate the complex natural conditions in which these consortia typically thrive.

To address these challenges, recent PROMICON studies have focused on how cyanobacteria interact with purple nonsulfur bacteria (PNSB). These bacteria, including Rhodopseudomonas palustris (*R. palustris*), have shown potential in producing biohydrogen and lipids by capturing nitrogen in oxygen-free environments. Nevertheless, a key limitation is that they need a carbon-based food source (e.g., acetate) to produce energy. A promising approach to overcome this issue involves growing *R. palustris* with cyanobacteria, which can pull carbon dioxide from the air and turn it into the organic carbon that *R. palustris* needs to thrive.

EVIDENCE AND ANALYSIS

To help turn greenhouse gases into sustainable energy and promote a circular bioeconomy, researchers in the international EU-funded PROMICON project successfully built and controlled a light-powered microbial community that produces hydrogen (H_2) and fatty acids by capturing carbon and nitrogen from the air. This highlights the potential of controlling and optimising phototrophic communities for sustainable bioenergy production. The full extent of the work and results described below are available in Pan, M. *et al.* (2025) and Roussou, S *et al.* (2025).

- Elemental balance confirmed that **the consortium successfully captured carbon and fixed nitrogen**.
- The strategy of controlling light cycles helped maintain low oxygen levels in the system, **allowing *R. palustris* to use nitrogen, even though oxygen is produced by *Synechocystis***.
- When infrared light was added to the light cycles, **the production of hydrogen (H_2) and fatty acids**, particularly C16 and C18, **increased significantly**.
- Proteomic analysis showed that the microbes exchanged acetate and **regulated their metabolic activities based on light**.
- **Infrared light boosted the production** of proteins involved in nitrogen fixation, carbohydrate processing, and transport in *R. palustris*, while **constant white light promoted the production of photosynthesis-related proteins** in *Synechocystis_acs*.

This study explores biotechnology that fixes atmospheric N_2 and CO_2, offering a sustainable path toward a net-zero emissions economy. However, challenges remain. Inactivating hydrogenases could prevent the consumption of produced H_2, and continuous reactors with selective membranes might improve performance. Long-term stability needs further testing.

POLICY IMPLICATIONS

The development and application of synthetic phototrophic microbial consortia for bioenergy production holds significant policy impacts, particularly in the context of the EU's sustainability and climate goals. The bioeconomy is stipulated by several EU policies and initiatives, including the Bioeconomy Strategy and the Circular Economy Action Plan.

- Phototrophic microorganisms hold great promise for capturing solar energy and converting greenhouse gases into sustainable energy carriers, providing an alternative solution to the increasingly fierce energy challenge.
- By harnessing the power of genetic engineering and light regulation, these synthetic microbial communities have the potential to revolutionise bioenergy production by enhancing the production of both biofuels and valuable chemicals through carbon capture.
- Such fatty acids-derived biofuels (e.g., via transesterification and esterification) even have a higher energy density and are more compatible with current infrastructure when compared to other forms of renewable energies.
- This process offers a sustainable alternative to traditional energy sources and directly supports key EU policies aimed at reducing carbon emissions and promoting renewable energy.
- Providing funding for genetic engineering and biotechnological research to optimise microbial strains for hydrogen production.
- Supporting the development of advanced bioreactors with selective membranes to enhance continuous H_2 and O_2 removal, improving efficiency and scalability.
- Establishing regulatory incentives and funding for long-term studies on N_2 and CO_2 fixation to ensure the viability of microbial systems for industrial applications.

SUSTAINABILITY AND LEGACY

The methodology, results and implications of the described work are openly available at https://doi.org/10.1021/acs.est.4c08629 and https://doi.org/10.1016/j.ymben.2025.01.008.

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PROJECT IDENTITY

PROMICON

Project name

Harnessing the power of nature through **PRO**ductive **MI**crobial **CON**sortia in biotechnology – Measure, model, master

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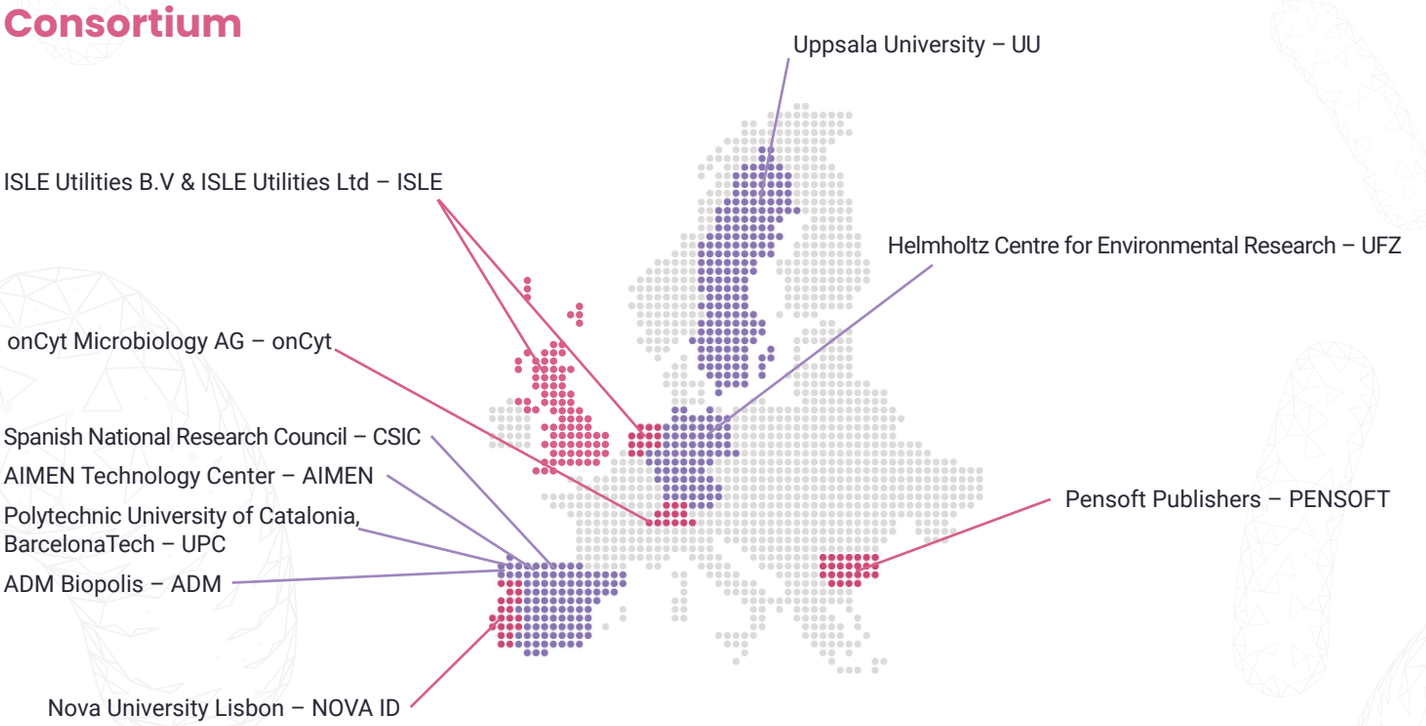
Duration

1 June 2021 – 31 May 2025 (48 months)

Consortium



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