

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

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 of-

fline 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.

PROJECT OBJECTIVES AND METHODOLOGY

The overall objective of the PROMICON project is 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 will achieve this by developing novel analysis and modelling approaches targeting essential key species in productive microbiomes, as well as whole microbiomes. PROMICON also aims to establish a standardised platform for obtaining quantitative single-cell data and connected coherent OMICS and Meta-OMICS data sets for complex microbiomes.

The methodology involves the analysis and modelling of both essential key species in productive microbiomes and whole microbiomes using a combination of data mining tools, mechanistic process models, and machine learning and deep learning approaches. The project also employs synthetic biology and systems metabolic engineering to optimise and assemble bacterial farmers, producers, and stabilisers, providing optimal production of target metabolites. The project focuses on developing sustainable bioproducts that can contribute to the circular economy, thus aligning with the objectives of the EU 2018 bioeconomy strategy.

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>.

PROJECT IDENTITY

PROMICON

Project name

Harnessing the power of nature through
PROductive **MI**crobial **CON**sortia in biotechnology –
measure, model, master

Coordinator and contact

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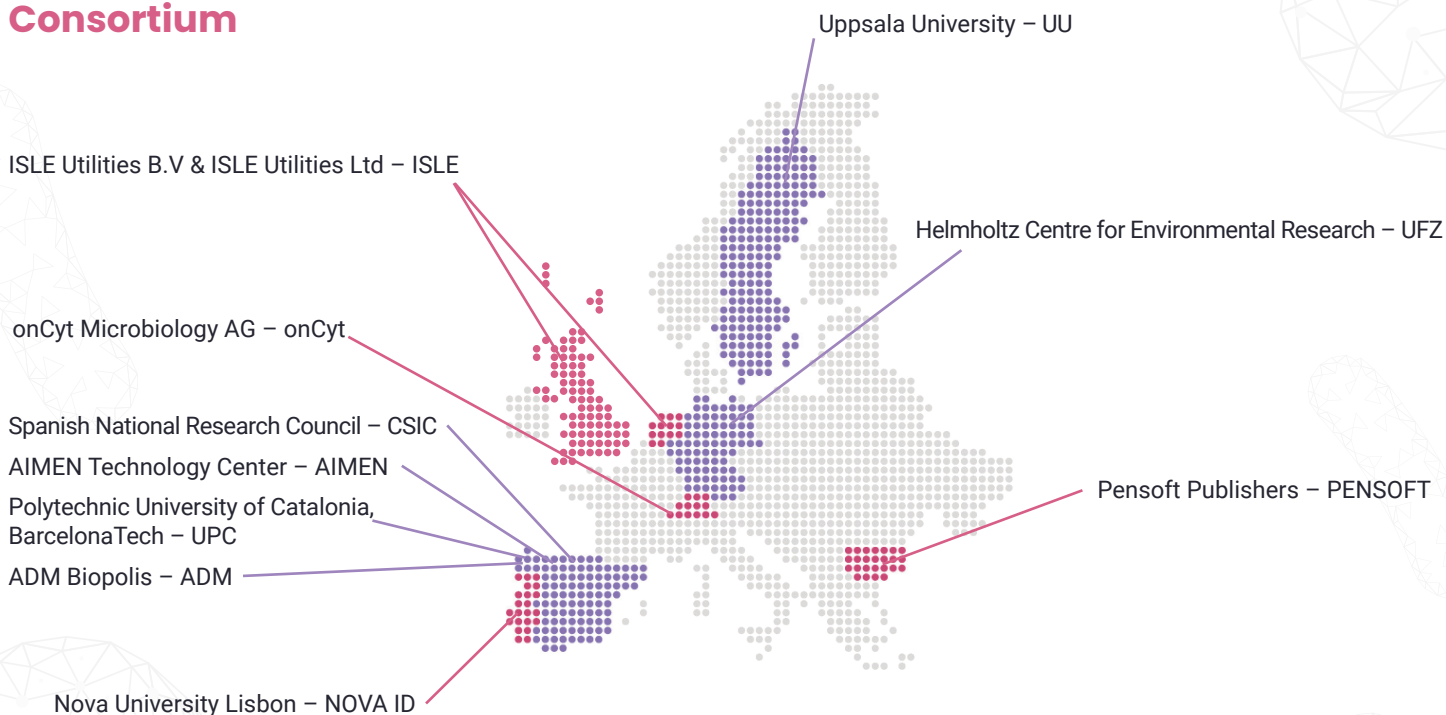
in [promiconh2020](https://www.linkedin.com/company/promiconh2020)

▶ [PROMICON](https://www.youtube.com/channel/UC...)

Duration

📅 1 June 2021 – 31 May 2025 (48 months)

Consortium



Budget

EU contribution: 5 999 712,50 €

Funding scheme

Call: Food and Natural Resources (H2020-FNR-2020)
Topic title and ID: FNR-12-2020 - Industrial microbiomes –
learning from nature

Further reading

● López-Gálvez, J. *et al.* (2022). *Running on-line single cell analysis platform*. Deliverable D1.4 EU Horizon 2020 PROMICON Project, Grant agreement No 101000733

● 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, 1559. <https://doi.org/10.3390/cells12121559>

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