From agro-food waste to biostimulant bacterial exopolysaccharides: solid-state fermentation as a circular bioeconomy tool

E. Garcia-Muchart[1], L. Mejias[1], N. Peña[1], O. Martínez-Avila[1], S. Ponsá[1]
[1]BETA Technological Centre, University of Vic-UCC, Vic, Catalonia, 08500, Spain
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Presenting author email: enric.garcia.muchart@uvic.cat

Introduction

Feeding a rapidly growing population has become a global priority. Efficient land use, yield improvement, quality production, and reducing agriculture's environmental and social impacts have become essential. While conventional fertilizers have contributed, they often harm the environment, crops, and soils. Biofertilizers from sustainable sources have gained importance, although their characteristics sometimes limit nutrient absorption by plants (Halpern et al., 2015). The use of biostimulants has notably increased in this context. These substances enhance nutrient uptake, plant growth, stress tolerance, and crop quality (du Jardin, 2012). Defined by function rather than composition, biostimulants include humic substances, protein hydrolysates, botanical extracts, chitin-based polymers, inorganic compounds, and beneficial microbes. Among these, bacterial exopolysaccharides (EPS), a polymeric biofilm produced by some bacteria under specific conditions provide interesting traits such as biosorption, biodegradability, water retention, and stress resistance properties (More et al., 2014). EPS production is well-established in the food and healthcare sectors, but it remains costly due to the dependence on expensive raw materials like sucrose and glucose.

An attractive option to make EPS production economically and sustainably is the use of agro-food by-products as low-cost substrates, providing an adequate sugar source and reducing final costs (Joulak et al., 2022). Moreover, this option offers a new perspective in the agro-food industry's value chain by introducing it into a circular bioeconomy framework.

In pursuit of sustainable and economically viable production, solid-state fermentation (SSF) is a promising technology for producing value-added industrial bioproducts such as biopesticides, bioplastics, enzymes, and aromatic compounds. Bacterial EPS has also been produced through SSF using fruit and grain substrates, achieving up to 61.4 mg of EPS per gram of dry matter (Garcia-Muchart et al., 2024; Stredansky et al., 1999). SSF is characterized by its low water and energy consumption, high productivity, waste reduction, and competitive advantages over conventional submerged fermentation in specific applications (Soccol et al., 2017).

Previous studies have been focused on identifying the strain-substrate best combination to produce EPS biostimulant through SSF and optimize this bioprocess at a lab scale. Thus, the objectives of the current work are to achieve a robust and reproducible operation at a higher bench scale, conduct a prospective sustainability assessment, and test the agronomic performances of the biostimulant obtained. This work aims to offer a new perspective in the agro-food value chain from three different perspectives: the biostimulant EPS production, the sustainability assessment of the process, and the efficiency of the biostimulant as an agronomic bioproduct.

Materials and methods

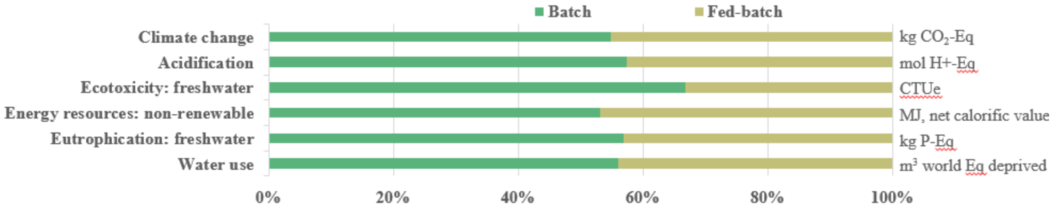
The bench-scale fermentation systems consisted of a dynamic respirometer with 6 L packed-bed PVC reactors connected to a mass flow controller (Bronkhorst High-Tech) that forced air through the solid bed of the reactor. The air was humidified before entering the reactor to prevent sample desiccation during fermentation. The outgoing air passed through a water trap to an oxygen sensor (O2-A2, Alphasense), and a data logging system (Arduino® and LabVIEW) collected the oxygen concentration. From these data, the specific oxygen uptake rate (sOUR) and cumulative oxygen consumption (COC) are calculated. Fermentation progress was also tracked through physicochemical analyses, including EPS production, carbohydrate quantification, reducing sugar consumption, biomass growth, pH variation, dry and organic matter content, and carbon-nitrogen ratio.

Results and discussion

Following the previous screening and optimization results (Garcia-Muchart et al., 2024), scaling up was carried out using *Burkholderia cepacia* as the selected bacterial strain with 30 times more GJW while maintaining the optimal SSF found conditions, except for temperature control. At this bench-scale, fermentation progressed similarly to the laboratory setup, reaching maximum temperatures of 45ºC, coinciding with peak oxygen consumption, bacterial growth, and sugar consumption. This system produced up to 73 mg g^{-1} DM of EPS over five days, suggesting that production and productivity remained consistent despite the larger substrate volume, lack of temperature control, and the use of a sanitized (but non-sterile) system.

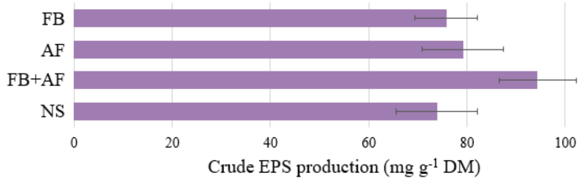
The same set of bench-scale trials provided data on energy, water, and reagent consumption, as well as emissions, enabling a prospective life cycle analysis of the system. Comparing a batch process with a fed-batch process revealed 10–15% lower impacts in the fed-batch scenario (Figure 1), driven by reduced water and energy consumption, primarily associated with inoculum preparation and the air compressor, two key *hotspots* identified in the analysis. Autoclaving the used substrates to sterilize them was detected also as a relevant point of the process considering the energy consumption.

Figure 1. Relative impact assessment of batch and fed-batch scenarios by categories



Thus, aiming to demonstrate the robustness of the bioprocess at this higher scale, different operation strategies to perform the SSF were compared considering their environmental and economic impacts and the productivity of EPS biostimulant. Using fed-batch (FB), reducing the airflow (AF), the combination of both (FB+AF), and non-sterilizing the substrate (NS) were the four operational strategies compared. Figure 2 shows that the less impactful conditions had the same productivity results than the others, meaning a promising perspective for an industrial approach of the bioprocess and its economy.

Figure 2. Crude EPS productivity of different strategies applied to reduce the impact of the process



Finally, the EPS were tested as an additive in agronomic trials to evaluate their potential as a biostimulant. Results showed significant improvements in plant performance and nutrient uptake under saline stress conditions.

Conclusions

This work presents evidence suggesting that using SSF with specific agro-industrial residues is effective for producing bacterial EPS with biostimulant properties within a circular bioeconomy approach. Following an initial screening of strains and substrates performed in previous studies, the GJW/*B. cepacia* combination emerged as the most promising for laboratory scale and in this work it was confirmed to be productive at a higher bench-scale. In both cases, productions of 72–73 mg g^{-1} DM were achieved over five days, demonstrating good scalability and reproducibility, even using restrictive operation conditions to reduce the environmental end economic impacts of the SSF process. It also was analyzed from an efficiency and a life cycle perspective, identifying key factors such as water consumption due to inoculum preparation and energy use by the compressor and the autoclave, which could be reduced through the implementation of a fed-batch strategy.

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