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I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM • XV SHEB • XV ENZITEC FLORIANÓPOLIS • 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
BIORREFINERY, BIOECONOMY AND CIRCULARITY

ELECTROFERMENTATION OF GLYCEROL BY A *Pseudomonas aeruginosa* STRAIN FOR 1,3-PROPANEDIOL PRODUCTION

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ABSTRACT

This study compared the conventional fermentation process with the electrofermentation approach for the production of 1,3-propanediol (1,3-PDO), a product currently classified as one of the most important building blocks for replacing petrochemicals. Using an unusual 1,3-PDO producer, the *Pseudomonas aeruginosa* strain EL14, and glycerol as the substrate, electrofermentation assays were conducted in a microbial electrochemical system under a constant potential of - 0.4V. The control assays were conducted in a conventional fermentation system, i.e., without an applied potential. The results indicated significant differences between the two approaches: electrofermentation increases significantly the concentration, the productivity, and the yield of 1,3-PDO at 87.6 ± 0.3 mmol L^{-1}, 8.67 ± 0.24 mmol L^{-1} h^{-1}, and 0.87 ± 0.17 mol 1,3-PDO mol^{-1} glycerol, compared to the control 49,6 ± 1.4 mmol L^{-1}, 4,96 ± 0.14 mmol L^{-1}, and 0,45 ± 0,02 mol 1,3-PDO mol^{-1} glycerol, respectively. This work demonstrates that electrofermentation can to enhance 1,3-PDO production from glycerol by *P. aeruginosa* EL14, offering a sustainable opportunity to the production of an industrially relevant compound.

Keywords: Electrofermentation. Fermentation. 1,3-PDO. Glycerol. *Pseudomonas aeruginosa* EL14.

1 INTRODUCTION

Electrofermentation represents an innovative approach that combines the fundamentals of traditional fermentation with electrochemical techniques, aiming to stimulate microbial metabolism through the electrochemical modulation of the intracellular redox state.[1] This technique uses electric current supplied by electrodes to influence the fermentation flux, regulating the oxidation-reduction potential (ORP), and the $NAD^+/NADH+H^+$ ratio.[2] The growing interest in electrofermentation is driven by its ability to enhance the production of valuable compounds, such as 1,3-propanediol (1,3-PDO). 1,3-PDO plays a crucial role in the chemical industry, being used as a monomer in the manufacture of polymers, particularly in the production of polytrimethylene terephthalate (PTT), a fiber with superior properties compared to traditional fibers like nylon and polyester. Currently produced via petrochemical routes, 1,3-PDO can be obtained biologically from glycerol fermentation by the genus *Clostridium*, *Citrobacter*, and *Klebsiella*. Works that mention *Pseudomonas* as 1,3-PDO producer are rare. Thus, glycerol fermentation for 1,3-PDO production represents a strategic research area, aligned with the pursuit of more sustainable processes in biorefineries, contributing to a circular and environmentally conscious economy. In this context, this study conducted the electrofermentation of glycerol by a *P. aeruginosa* strain, to the efficient conversion of glycerol into 1,3-PDO, a key component in replacing petrochemicals.

2 MATERIAL & METHODS

P. aeruginosa EL14 was previously isolated from a mixed consortium of an electrogenic biofilm acclimated with glycerol in a Microbial Fuel Cell, and its electroactivity and ability to produce 1,3-PDO have been reported[3]. The genome of EL14 is available at NCBI under the GenBank accession number JASMRB000000000. The M9 culture medium (NaCl 1.0 g L^{-1}, NH_4Cl 1.0 g L^{-1}, $MgSO_4$ 0.25 g L^{-1}, K_2HPO_4 13.53 g L^{-1}, KH_2PO_4 3.03 g L^{-1}, yeast extract 1.0 g L^{-1}, and glycerol 10 g L^{-1}) was used in both fermentation and electrofermentation assays. To remove dissolved oxygen from the culture medium and in the fermentation flasks, N_2 was sparged for 10 minutes. Conventional fermentation was carried out in 100 mL penicillin-type flasks, and electrofermentation was conducted in 125 mL single-chamber bioelectrochemical systems, with a set of two electrodes: a platinum wire electrode and a carbon cloth electrode (9 cm^2) suspended by a nickel-chromium wire for external connections. The electrofermentor was connected to a regulated power supply (Hikari, HF-3205S) set to maintain a constant potential of - 0.4 V during the assay. The assays were conducted in triplicate at 37°C and 150 rpm. The initial optical density at 600nm (OD_{600}) was standardized to 0.1, and samples were collected every 2 hours for the determination of OD, pH, substrate, and products. Quantifications of substrate and products were performed by high-performance liquid chromatography (HPLC) using an LC-20 AT chromatograph (Shimadzu, Japan) equipped with an Aminex HPX-87H column. The column temperature was maintained at 60°C, with a mobile phase of 5 mmol L^{-1} H_2SO_4 at a flow rate of 0.6 mL min^{-1} (84 kgf cm^{-2}). The detectors used were a refractive index detector (RID) for glycerol and 1,3-PDO, and a photodiode array (PDA) detector at 210 nm for organic acids.

3 RESULTS & DISCUSSION

The concentration of 1,3-PDO from glycerol by *P. aeruginosa* EL14 was compared in a conventional fermentation process (FE) and electrofermentation (EF). Cell growth (OD_{600}), substrate, products, and pH over time are illustrated in Figure 1. In conventional fermentation, the lag phase of cell growth lasted 2 hours, whereas, in electrofermentation, this phase was not observed.

Additionally, in EF, the exponential phase was more prolonged, resulting in higher cell growth, with an OD_{600} of 3.40 ± 0.05, in contrast, the maximum OD_{600} in conventional fermentation was 2.30 ± 0.04. Substrate consumption (Figure 1B) shows an interesting aspect: in EF, the substrate was not completely consumed, although cell growth was higher compared to FE, where the substrate was completely consumed. This, could be probably attributed to the pH in EF that decreases until 6.4 due to the acetic acid formation. These results indicate that the electrical potential in EF acted as an additional energy source, allowing the cells to benefit from the extra energy input for growth and maintenance.

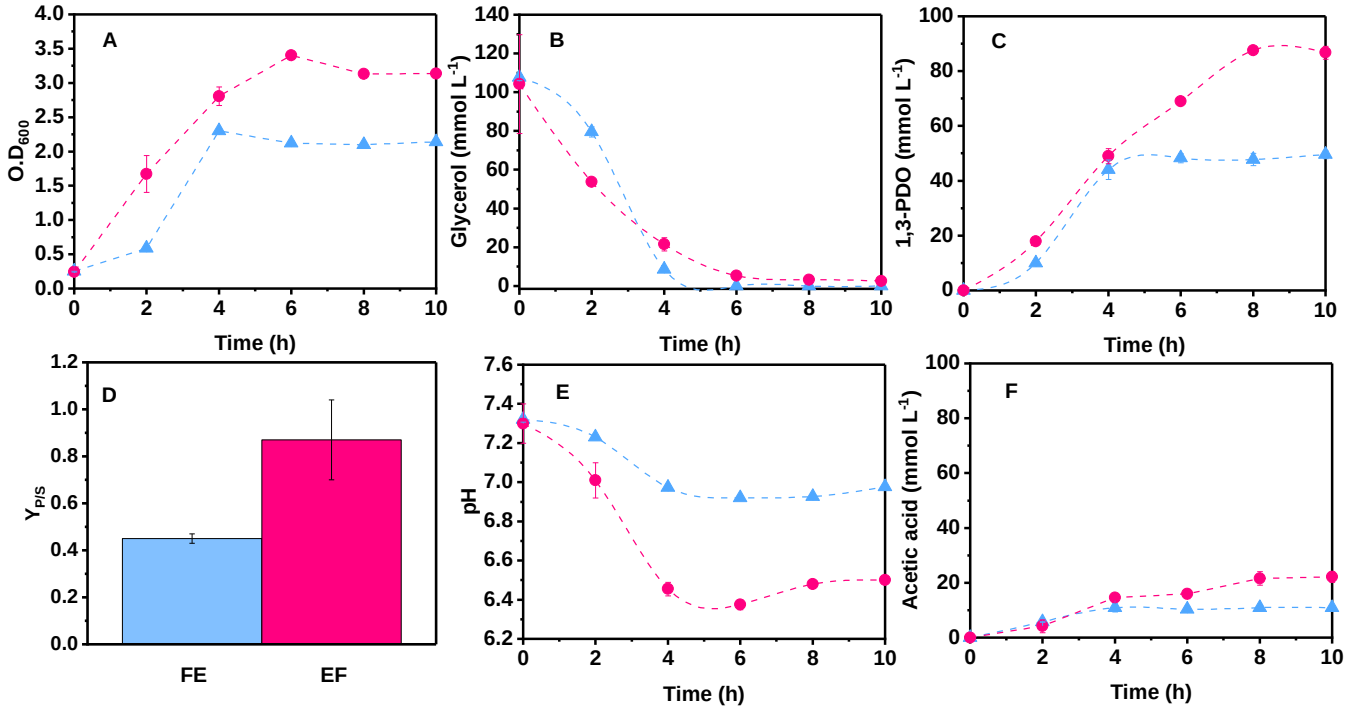

Figure 1 conventional fermentation assays (blue line) and electrofermentation (pink line). A) OD_{600}; B) Glycerol; C) 1,3-PDO; D) $Y_{P/S}$ (mol 1,3-PDO mol^{-1} glycerol); E) pH; F) Acetic acid. Error bars represent the standard deviations among triplicates.

The 1,3-PDO was significantly higher in EF, reaching 87.6 ± 0.3 mmol L^{-1}, while in FE the maximum attained was 49.6 ± 1.4 mmol L^{-1} (Figure 1C). Additionally, the productivity was 4.96 ± 0.14 mmol L^{-1} h^{-1} in FE compared to 8.67 ± 0.24 mmol L^{-1} h^{-1} in EF, indicating that the electrofermentative also accelerated the 1,3-PDO formation rate. The substrate-to-product conversion factor ($Y_{P/S}$) (Figure 1D), which reflects the amount of glycerol destined for 1,3-PDO production, also showed that the applied potential directed glycerol metabolism towards the reductive pathway where 1,3-PDO formation occurs. The yield was estimated at 0.45 ± 0.02 mol 1,3-PDO mol^{-1} glycerol in FE and 0.87 ± 0.17 in EF. This result may be associated with the alteration of intracellular ORP by the application of a negative potential of - 0.4 V, which likely triggered an increase in reduced cofactor $NADH+H^+$, favoring the reductive pathway of glycerol metabolism responsible for regenerating NAD^+ consumed in the oxidative pathway, thus maintaining redox balance. Acetic acid (Figure 1F), a product of the oxidative branch, was obtained at 10.94 and 22.14 mmol L^{-1} in FE and EF, respectively. The increase in acetic acid in EF is associated with the increased of 1,3-PDO, as aforementioned, the oxidative and reductive pathways are interdependent and operate in a coordinated manner to preserve redox homeostasis. Finally, the decrease in pH during fermentation and electrofermentation assays (Figure 1E) was attributed to acetic acid production.

Table 1 Concentration of 1,3-PDO, yield, and productivity in fermentation and electrofermentation assays.

Approach	1,3-PDO (mmol L^{-1})	$Y_{P/S}$ (mol mol^{-1})	Productivity (mmol L^{-1} h^{-1})
Fermentation	49.6 ± 1.4	0.45 ± 0.02	4.96 ± 0.14
Electrofermentation	87.6 ± 0.3	0.87 ± 0.17	8.67 ± 0.24

4 CONCLUSION

The results demonstrated that the electrofermentation of glycerol by *P. aeruginosa* EL14 is a promising approach for 1,3-PDO production, surpassing conventional fermentation in terms of 1,3-PDO concentration, productivity, and yield. The potential of - 0.4 V was adequate to improve 1,3-PDO production, but other potentials can be tested. Thus, electrofermentation represents an opportunity for glycerol conversion into 1,3-PDO, promoting more sustainable and efficient processes.

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ACKNOWLEDGEMENTS

The authors would like to thank the National Council for Scientific and Technological Development (CNPq) for granting a scholarship to the first author (167515/2022-5), and the São Paulo Research Foundation (FAPESP) for financial support for this study (2022/040024-0).