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Electrochemical startup increases 1,3-propanediol titers in mixed-culture glycerol fermentations

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ABSTRACT

In this study we investigated the use of electric potential to bioelectrochemically ferment glycerol, a cheap by-product of biodiesel production, into valuable 1,3-propanediol (1,3-PDO). The 1,3-PDO production rates were increased up to 6 times in electrofermentations, compared to non-electrochemical fermentations, and high concentrations up to 42 g 1,3-PDO/l were achieved in fed-batch mode. Extensive growth of the well-known 1,3-PDO producers *Clostridiaceae* (55–57%) was observed when an appropriate potential (−1.1 V vs. SHE) was constantly applied since the start. Potential propionate producers (*Veillonellaceae*) were also among the dominant families (20–21%); however, surprisingly enough, propionate production was not observed. On the contrary, *Clostridiaceae* were absent, *Veillonellaceae* dominated (56–72%), and propionate was produced when electric potential was not sufficient for current production since the beginning. In all cases, glycerol consumption ceased and electrocatalytic activity was lost when we replaced the biofilm electrodes with electrodes lacking a biofilm, clearly demonstrating that glycerol electrofermentation was mostly supported by the bacteria located in the biofilm. In the non-electrochemical systems the performance and the titers achieved were poor; only 18 g 1,3-PDO/l was achieved in more than twice the time, and lactate producing *Lactobacillaceae* became dominant.

1. Introduction

The biorefinery is an alternative to the use of fossil fuels where energy and commodity chemicals are sustainably produced using alternative chemistry processes [1]. Recently, this new concept has attracted a lot of attention from policy makers, research institutes, and the industry [1]. One major process that emerged from this paradigm shift in energy production is the production of biodiesel, which has already exceeded the production of 6 billion liters globally [1]. Biodiesel is produced from the transesterification of triglycerides, using methanol and sodium hydroxide as a catalyst [2]. Apart from the unreacted methanol, a major by-product of the process is glycerol; approximately 1 l of glycerol is produced per 10 l of biodiesel [2], which has resulted in increasing amounts of glycerol produced every year. On the other hand, industrial demands for glycerol did not increase accordingly and glycerol's market price dropped substantially, forcing the closure of a number of glycerol producing plants [3].

A number of value-added products can be produced during glycerol fermentation, like for example hydrogen, ethanol and succinate [4]. Amongst them is 1,3-propanediol (1,3-PDO), a product with an expanding market and a continuously increasing demand of over 50,000 tons per year, which has attracted a great commercial interest because of its extensive use in the chemical industry (e.g. for polymer synthesis, cosmetics, solvents, as an antifreeze, and in lubricants) [4,5].

Bioelectrochemical systems (BES), which employ microbial "catalysts" on electrodes to facilitate electrochemical reactions, have been tested for improving the rates and yields of glycerol conversion. However, the number of studies with glycerol electrofermentations in the cathode remains limited [4,6–8]. Selembo et al. [8] were the first ones to employ polarized anodes and cathodes in single-chamber, batch operating glycerol fermentations, and managed to increase the hydrogen yields produced by conventional glycerol fermentations. Later on, Dennis et al. [7] studied the metabolites produced during continuous, bioelectrochemically-altered glycerol fermentations, in association with the microbial population shifts. Interesting microbial correlations were obtained, showing the relationship between the metabolic products and the microbial population shifts. However, 1,3-PDO production was not the main metabolic product in this study, and the application of

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electrical current did not affect 1,3-PDO production in a positive way. The first study which clearly demonstrated an increased 1,3-PDO production was that of Zhou et al. [4] who used batch biocathodes to study the carbon and electron fluxes during bioelectrochemically enhanced glycerol fermentations. In a more recent study, Choi et al. [6] used pure cultures of *Clostridium pasteurianum* to demonstrate a successful shift in the microbial metabolism toward enhanced 1,3-PDO production when electrical potential is supplied. Improved 1,3-PDO production was demonstrated in both these last two studies; however, it was not the authors' aim to maximize 1,3-PDO concentrations and the systems operated at relatively low 1,3-PDO concentrations (up to 7.22 g/l in Choi et al.).

Extracting 1,3-PDO at low concentrations from the fermentation streams will be costly and ineffective, but on the other hand high 1,3-PDO concentrations can have an inhibitory effect on the microbial populations [9,10]. Production of 1,3-PDO at relatively high concentrations is possible by pure cultures of bacteria like *Enterobacteriaceae* [11] and *Clostridiaceae* [12]. However, using pure cultures will imply considerably higher costs related to avoiding contamination in the bioreactors, and therefore using mixed cultures could be beneficial [7,13,14]. Another argument in favor of using mixed or co-culture populations is that the symbiotic relationships that evolve can have a positive effect not expressed by the individuals [15]; in bioelectrochemical systems, this can result in higher electrical current produced [16], which could be beneficial for enhancing 1,3-PDO production. Despite these arguments, product specificity will be hard to achieve when using mixed cultures, and industrialization of the electrofermentation technology will most likely require defined pure or co-cultures of bacteria. In any case though, identifying the bacteria or bacterial combinations that will be used in this relatively new for the industry technology, is a question that could be approached by investigating mixed bacterial cultures. This will be particularly important to understand the needs and capabilities of these bioelectrochemically-modified environments, and therefore to further optimize their performance.

Up until now, the bacterial species that thrive in mixed culture electrofermentations where 1,3-PDO is the main metabolite produced at high concentrations have not been disclosed. In addition, the effect of electric potential on the performance and bacterial composition has only fairly been studied. In this study we aimed at bioelectrochemically enhancing 1,3-PDO production from glycerol, and we did this by studying glycerol-fermenting biocathodes under different electrochemical conditions and in fed-batch mode. After increasing 1,3-PDO to the highest concentrations reported in a glycerol electrofermentation study, we attempted a deeper insight into the process by investigating how the application of different electrochemical conditions affected the bacterial population, in relation to the different metabolites produced. Finally, cyclic voltammetry analysis allowed us to better acknowledge the effect of the applied conditions on the electrocatalytic activity of the glycerol fermenting biofilm.

2. Materials and methods

2.1. Reactor construction

Dual-chamber, H-type borosilicate reactors with a working volume of 260 mL in each chamber were employed to study the effect of cathodic current evolution on glycerol fermentations. The chambers were separated using a cation exchange membrane (CMI-7000, Membranes International Inc., USA) and reactors were assembled as described in Xafenias and Mapelli [17]. The electrodes were made of graphite felt (SIGRATHERM; SGL Carbon Ltd., UK) and were constructed and pre-treated as described elsewhere [18], with the exception that 0.8 mm titanium wires were used instead of copper, to reinforce the electrodes' resistance to corrosion. New electrodes were prepared for each experiment, with a total projected surface area of 38 cm^2 unless otherwise indicated. In order to avoid the counter electrode (CE) being current limiting, a larger electrode surface area was used in the CE chamber by immersing the 38 cm^2 electrode into a chamber containing 30 pieces of graphite felt with dimensions of 1.5 cm × 2.0 cm × 0.5 cm each. Biological non-electrochemical (NE) experiments were carried out in single borosilicate bottles lacking any electrodes.

2.2. Media and inoculum

A phosphate-buffered mineral medium [19] was used (pH 7.3 which drops to pH 6.6 when CO_2 saturated) in both the working electrode (WE) and the CE chambers of the H-type reactors, and in the control NE reactors. Biotechnology grade glycerol (99%; Amresco Inc., USA) was pulse-fed to give maximum glycerol concentrations of 11.0 ± 1.7 g glycerol/l in the WE chambers of the bioelectrochemical reactors and in the control reactors as noted, whenever concentration was lower than the average value of 0.8 g glycerol/l. When mentioned, pH adjustments were made by manual addition of 5 M NaOH.

The mixed microbial consortium used to inoculate the reactors originated from the anaerobic mesophilic (37 °C) sludge treatment process of Gothenburg's wastewater treatment plant (Gryaab AB, Sweden), and was stored for a period of 6 months at 4 °C prior to use. The bacterial composition of inoculum from the same source has been analyzed previously [17].

2.3. Setup and operation

Three different bioelectrochemical setups were studied, all with polarized WE immersed in the inoculated glycerol-containing medium. In order to maintain anaerobic conditions and to balance the pH rise caused by cathodic current production, the medium was continuously sparged with CO_2. In the first setup (fixed potential; FP), duplicates of electrodes polarized at potentials of −1.10 V operated for 15 days, after which both electrodes and part of the suspension were removed for microbial community analysis. In the second setup (fixed potential-increased electrode surface area; FP-ISA), the WE was in contact with 20 pieces of graphite felt (1.5 cm × 2.0 cm × 0.5 cm) to test the effect of higher current produced under the same potential of −1.10 V. The third setup (varying potential; VP) was a control setup with electrodes, which tested in duplicates whether decreasing the electrode potential from −0.80 V to −1.10 V stepwise would improve the system's performance. In this setup the electrodes were polarized at a starting potential of −0.80 V for the first 19 days and from then on at −0.90 V for 10 days, −0.95 V for 8 days, −1.00 V for 3 days, −1.05 V for 3 days, and −1.10 V for 4 days. At the end of operation the WE of the FP-ISA and the VP reactors were replaced with new ones lacking a biofilm and the experiments were prolonged for another 7 days. This was to test whether current produced under the same potential but with no established biofilm would alter the performance of the system. Additionally, two non-electrochemical setups, one sparged with CO_2 (NE-CO_2) and the other one with N_2 (NE-N_2), were inoculated from the same source and had the same medium as the electrochemical reactors, but ran in the absence of electrodes. Adding electrodes in open circuit would not represent an appropriate control of the FP reactors because of the absence of electrostatic interactions with the planktonic biomass and the medium; in this aspect, the VP reactors were considered more appropriate, and the NE reactors represented a more conventional fermentation system without any electrodes. All reactors employed in this study were covered with aluminum foil to

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