Driving the fermentation patterns by redox potential control using Bio-Electrochemical Systems (BES)

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Abstract
In a context of environmental biorefinery, *i.e.* using various organic resources from wastes or agricultural residues, driving the fermentation end products of anaerobic mixed cultures using the few number of environmental parameters that could be changed (pH, temperature, OLR…) is rather a difficult issue. In this study, an electrochemical control of the redox potential was proposed as an alternative solution. The use of bioelectrochemical systems (BES) to control the redox potential in dark fermentation of glucose showed a two-fold increase of hydrogen production compared to conventional fermentation. When the same system was applied to glycerol fermentation, no significant difference was observed for 1,3-PDO production. Nonetheless, the addition of electro-active bacteria in BESs increased the 1,3-PDO production by 33% compared to conventional fermentation with performances close to the theoretical maximal value. The metabolic pattern in electro-fermentation after substrate depletion was significantly different from the conventional fermentation control, with, in all cases, a substantial decrease of unwanted lactate pathway.

Keywords
Bioelectrochemical system; Electro-fermentation; Dark Fermentation; Redox potential control; Biohydrogen

INTRODUCTION

Fermentation processes can be used to produce biomolecules of industrial interest from various organic substrates. As an example, glycerol, a byproduct of biodiesel production, can be fermented to 1,3-propanediol (PDO) and H_2 [1]. Furthermore, glucose has been widely used to study and optimize the metabolic pathways involved in H_2 production [2]. Overall, the control of the fermentation patterns is limited to only few operating parameters, mainly temperature, pH, hydraulic retention time (HRT) and organic loading rate (OLR). Anaerobic microorganisms and microbial activity in general are particularly sensitive to redox potential (ORP) changes. ORP affects intracellular redox homeostasis and consequently drives profiles of fermentation products and its regulation provides an alternative for better control of fermentation processes [3]. Different strategies can be used to adjust extracellular ORP including strains modification, supply of chemical reductive or oxidative agents, bubbling gases and electrochemical control of ORP [4]. The use of microbial bio-electrochemical systems (BESs) has been widely investigated over the last decade for microbial fuel cell applications [5]. However, electrochemical control of ORP in anaerobic process, such as methanogenesis [6], has recently opened a new and emerging research field.

The bioelectrochemical systems (BES) provide a way to apply an ORP with no addition of chemical agents that are often environmentally unfriendly. There are no range limits for applying an ORP in BESs contrary to reductive or oxidative chemical agents that can be toxic for bacteria [6]. BESs also provide additional electrons that can be used by electro-active bacteria such as *Geobacter sulfurreducens* and S*hewanella oneidensis*. These microorganisms are able to perform direct electron transfer (DET) with electrodes and other bacteria [7]. In this context, it is possible to consider consortia formed by fermentative and electro-active bacteria to maximize the electron recovery from substrate oxidation [8], [9].

The aim of this work was to investigate the effect of an electrochemical control of the ORP on fermentation patterns. Mixed culture fermentations were performed with glucose or glycerol as carbon and energy sources under non-sterile conditions.

MATERIAL&METHODS

Microorganisms

Inocula were mixed cultures issued from a continuous dark fermentation lab-scale reactor used for H_2 production from glycerol, and sludge from wastewater treatment plant of Narbonne, France, for glycerol and glucose experiments, respectively. *Geobacter sulfurreducens* strain DSM No. 12127 was purchased from DSMZ.

Culture and fermentation medium

The fermentation medium used for glycerol experiments was the same as Dietz et al. [13] with 100mM phosphate buffer and pure glycerol (>99,9%) at a concentration of 17.5g.L^{-1} as carbon source. The fermentation medium used for glucose experiments was adapted from Rafrafi et al. [10] using 5g.L^{-1} glucose and 100mM MES buffer. The *Geobacter* medium 826 (DSMZ) was used for the biofilm preculture on the graphite electrode.

Bioelectrochemical set-up and experiments

Batch experiments were run in duplicates in potentiostatically controlled half-cells containing 800 mL of solution with 200 mL of headspace. The two half-cells were separated with a cation exchange membrane (Fumasep® FAA-3-PK-130). Working electrodes for glycerol experiments were 2.5 cm*2.5 cm*0.25 cm planar graphite plates and counter electrodes were 90% platinum–10% iridium grids. All the electrodes used for glucose experiments were 90% platinum–10% iridium grids. In all experiments, the working electrode was set at a fixed applied potential of -650 mV *vs* SHE using a potentiostat/galvanostat VMP3 (BioLogic Science Instruments, France).

Metabolite and biogas analysis

Concentrations of glucose, glycerol, 1,3-PDO and organic acids were measured by HPLC with a refractive index detector (Waters R410). Biogas composition was determined using a gas chromatograph (Clarus 580, Perkin Elmer) equipped with a thermal conductivity detector.

Characterization of microbial communities

The microbial community structure was analyzed after DNA extraction and PCR amplification by Capillary Electrophoresis-Single Strand Conformation Polymorphism.

RESULTS AND DISCUSSIONS

Control of metabolic pathways in electro-fermentation process

The H_2 yield observed in electro-fermentation of glucose was 0.97±0.09 mol H_2 $mol^{-1}_{glucose}$, which is a 70% increase compared to conventional fermentation with a yield of 0.57±0.09 mol H_2 $mol^{-1}_{glucose}$. This yield is higher than those usually reported when mixed cultures are used with no pretreatment (between 0.2 – 0.7 mol H_2 $mol^{-1}_{glucose}$) [11]. Besides, a control was carried out with no inoculum and showed that no H_2 was electrochemically produced. This increase of H_2 production could be explained by a change in the microbial community structure and/or a modification of the redox balance in bacteria. First results from CE-SSCP analyses did not reveal significant differences between bacterial communities from conventional fermentation and electro-fermentation. Sequencing is under progress. This suggests a redirection of metabolic routes only by the applied potential. Fig.1A shows the COD balance calculated for glucose fermentation. The applied voltage caused a significant decrease in lactate production, driving the metabolic pathway towards the production of butyrate and H_2. This link between high H_2 production and butyrate concentration was previously reported by Rafrafi et al.[10].

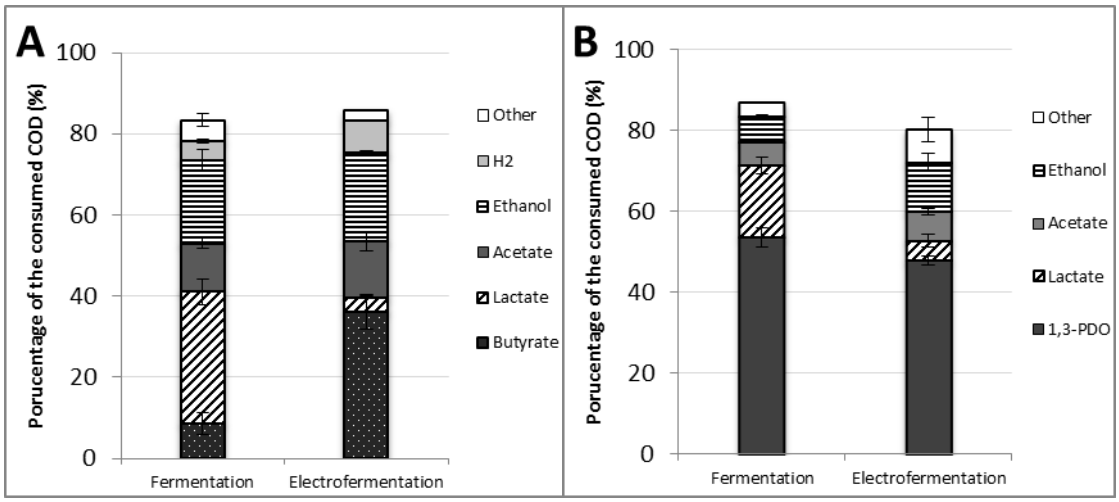

Figure 1 COD balances calculated from the metabolites measured at the end of fermentation in fermentation and electro-fermentation of glucose (A) and glycerol (B) with resp. no redox potential applied and an applied redox potential of -650mV *vs* SHE. Results are normalized on the consumed COD. H_2 was only measured for glucose experiments. "Others" refers to formate, succinate and propionate.

In the case of glycerol fermentation, 1,3-PDO yields after conventional fermentation (0.47±0.00 $mol_{1,3\text{-}PDO}$ $mol^{-1}_{glycerol}$) and electro-fermentation (0.42±0.01 $mol_{1,3\text{-}PDO}$ $mol^{-1}_{glycerol}$) were not significantly different. Similar results were obtained by Zhou et al. [12], when they studied the effect of an ORP of -600mV *vs* SHE in a BES with mixed culture. The COD balances after one week of fermentation are presented Fig.1B. As for glucose experiments, lactate production decreased when an ORP was applied. The global metabolism was redirected to ethanol and propionate production. Sequencing and analysis of bacterial community structures are under progress.

Electro-fermentation with engineered consortia

In order to increase the production of reduced molecules and favor electron transfer in the BESs during electro-fermentation, *Geobacter sulfurreducens* was first grown as a biofilm

on the working electrode before addition in the fermentation reactor. *G. sulfurreducens* is known for performing direct electron transfer (DET), consuming volatile fatty acids but not glycerol. Speers et al. [8] successfully used this electro-active microorganism to increase ethanol production by a strain of *Clostridium cellobioparum* while consuming by-products during glycerol fermentation. Electro-fermentation with electrodes colonized with *G. sulfurreducens* produced more 1,3-PDO with a yield of 0.62±0.07 $mol_{1,3\text{-}PDO}$ $mol^{-1}_{glycerol}$ which is 32% higher than the yield obtained in conventional fermentation. This yield is very close to the maximum theoretical yield of 0.71 $mol_{1,3\text{-}PDO}$ $mol^{-1}_{glycerol}$ [13]. To our knowledge, this is the highest yield obtained with mixed cultures. Furthermore, unwanted lactate production was reduced (~8% of consumed COD) compared to conventional fermentation. The mechanisms behind the decrease of lactate production during electro-fermentation are still unknown. The same experiment with glucose as substrate is under progress.

CONCLUSION

In our study, it was shown the possibility of using successfully BESs to redirect metabolic pathways and therefore control the fermentation patterns. More experiments are under progress to measure the real impact of the ORP on flexibility and stability of the fermentation process. By controlling ORP and electron transfer between fermentative and electroactive bacteria, it is expected not only to propose an alternative way for better fermentation control/optimization, but also to provide new insights in global metabolism related to electron transfer occurring in anaerobic mixed cultures.

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