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Stimulation of electro-fermentation in single-chamber microbial electrolysis cells driven by genetically engineered anode biofilms

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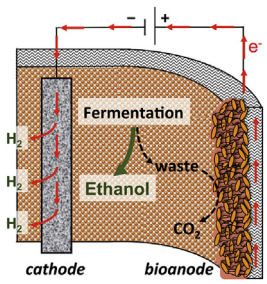
HIGHLIGHTS

- Fermentation reactors were fitted electrochemically as microbial electrolysis cells.
- A bioanode was designed to efficiently remove byproducts of ethanol fermentations.
- The bioanode stimulated fermentation and enriched for the ethanol product.
- Electro-fermentations show promise to enable novel fermentations and reduce processing costs.

GRAPHICAL ABSTRACT



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ABSTRACT

Unwanted metabolites produced during fermentations reduce titers and productivity and increase the cost of downstream purification of the targeted product. As a result, the economic feasibility of otherwise attractive fermentations is low. Using ethanol fermentation by the consolidated bioprocessing cellulolytic bacterium *Cellulomonas uda*, we demonstrate the effectiveness of anodic electro-fermentations at maximizing titers and productivity in a single-chamber microbial electrolysis cell (SCMEC) without the need for metabolic engineering of the fermentative microbe. The performance of the SCMEC platform relied on the genetic improvements of anode biofilms of the exoelectrogen *Geobacter sulfurreducens* that prevented the oxidation of cathodic hydrogen and improved lactate oxidation. Furthermore, a hybrid bioanode was designed that maximized the removal of organic acids in the fermentation broth. The targeted approach increased cellobiose consumption rates and ethanol titers, yields, and productivity three-fold or more, prevented pH imbalances and reduced batch-to-batch variability. In addition, the sugar substrate was fully consumed and ethanol was enriched in the broth during the electro-fermentation, simplifying its downstream purification. Such improvements and the possibility of scaling up SCMEC configurations highlight the potential of anodic electro-fermentations to stimulate fermentative bacteria beyond their natural capacity and to levels required for industrial implementation.

1. Introduction

Microbial fermentations have a long history in food production and conservation and, more recently, they have enabled the large-scale production of biofuels, chemicals, and pharmaceuticals [1]. The economic feasibility of industrial fermentations requires processes that reach titers and productivity high enough to reduce the

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cost of downstream processing [1]. To do this, strain and process improvements are often needed that maximize substrate consumption and reroute the fermentative metabolism towards the synthesis of the desired product and away from unwanted metabolites. This is not always attainable because microbial fermentations achieve redox balance by recycling reducing power in auxiliary reactions that often generate a mix of reduced products, such as alcohols, organic acids and H_2 gas, which the cell excretes. The formation of these unwanted metabolites in industrial-scale fermentations can lead to pH imbalances and/or feedback inhibition of the growth of the fermentative metabolism, increasing batch-to-batch variability and reducing productivity, titers and product quality [2]. As a result, extensive genetic engineering is often needed to reroute the natural fermentation pathways towards the targeted metabolite and increase product titers. This limits industrial scale fermentations to a narrow range of bacteria and yeasts that have the robustness and genetic amenability necessary to maximize the production of the targeted product [1]. Nearly all of the bioethanol fuel produced in the United States is, for example, produced through the fermentation of corn grain by industrial yeast strains, which have the robustness and stress tolerance required for optimal ethanol yields at large scales [3,4]. Yet waste glycerol is produced that diverts up to 4% of the fermentable sugar away from the alcohol product [5] and further reduces profit through the addition of a disposal fee [6]. Cellulosic ethanol production, on the other hand, is most efficient in bacterial fermentations [7], but several byproducts are also generated that reduce ethanol yields and can drop the pH, feedback inhibit the fermentation, and increase the cost of purification [5–7]. Glucose fermentation to ethanol by *Zimomonas mobilis* under anaerobic conditions generates, for example, acetoin, glycerol, acetate, and lactate byproducts [8]. *Escherichia coli*, on the other hand, diverts glucose, when provided at high loadings, towards acetate production, a pathway that does not consume reducing power but yields ATP [9].

Tapping into the many bacteria and fungi that carry out fermentations in nature shows promise for the development of novel fermentation-based technologies for biofuels [10]. However, strain improvement of environmental microbes is often challenging because their fermentative metabolism is naturally suited for cooperative interactions with partner organisms, which remove the fermentation products before they accumulate [11]. Organic acids such as acetate, formate, and lactate and alcohols such as ethanol produced in microbial fermentations can be, for example, fully oxidized to CO_2 by respiratory microbes that use terminal electron acceptors such as nitrate, iron and manganese oxides, and sulfate whereas methanogens can use H_2, formate and acetate to produce methane [11]. Such syntrophic interactions prevent the accumulation of feedback inhibitors of fermentation and stimulate the rates of biomass degradation [12–15]. Fermentations can also be stimulated electrochemically without syntrophic partners. A 1940 process, coined electro-fermentation, used electrical currents to accelerate the rates of fermentation of tea leaves and improved the taste and strength of the resulting product [16]. The term electro-fermentation is now used more broadly to include processes that use electrodes to modify the medium and influence the redox balance of a fermentation [17]. The electrodes can also be used as electron acceptors (anodic electro-fermentation) or donors (cathodic electro-fermentation) to overcome the metabolic limitations of balanced fermentative reactions and stimulate them beyond their natural capacity [17,18].

Bioelectrodes (electrodes colonized by electrochemically active microorganisms) can also be used to influence the titers and purity of specific fermentation products in electro-fermentations driven by undefined [19] and defined [20,21] cultures. Of particular importance for scaled up applications are anodic electro-fermentations driven by biofilms of the exoelectrogen *Geobacter sulfurreducens* [20–22]. This bacterium can conserve energy for growth by transferring electrons from common fermentation products such as acetate, formate, lactate and H_2 to electrodes [22–24]. When operated under potentiostatic control, as in a microbial electrolysis cell (MEC) [25–27], a differential potential is generated between the anode and the cathode that promotes the growth of the exoelectrogenic biofilm on the anode electrode [28]. The bioanode can efficiently remove fermentation byproducts as soon as they are produced, generating electricity and preventing pH imbalances and the accumulation of feedback inhibitors of the fermentation [20,21]. The applied potential also removes cathodic limitations and allows for the reduction of protons at the cathode and co-generation of H_2 as an added-value product [20] at yields much higher than those achieved fermentatively [29].

The implementation of anodic electro-fermentations at industrial scales will ultimately require the development of processing technologies that integrate and optimize electrochemistry, process engineering, fermentation technology and metabolic engineering. Standard H-type MECs are clearly not scalable. Yet standard fermentation reactors could be retrofitted with anode, cathode and reference electrodes to operate as MECs. Microbiological limitations must also be considered to improve the efficiency of metabolite removal and reduce electron losses at the anode. This can be accomplished by using bioanodes that interact synergistically with the fermentative partner so most, if not all, of the unwanted fermentation products are converted into electricity before they accumulate to inhibitory concentrations. The production of cathodic H_2 must also be considered as it can inhibit the growth of the fermentative microbe [30] and/or be reoxidized by the bioelectrode. Indeed, *G. sulfurreducens* oxidizes H_2 in MECs [23] and can simultaneously oxidize H_2 and acetate, though at the expense of reducing the efficiency of acetate oxidation [31]. This can lead to acetate accumulation in the broth and the competitive inhibition of sugar uptake [32] and of cellulase enzymes synthesis [33] by fermentative partners. Lastly, the fermentative strains and bioanodes must be robust enough to tolerate the high concentrations of substrates and/or added-value products that are relevant to large-scale electro-fermentations. Based on these considerations, we designed a single-chamber MEC (SCMEC) to demonstrate the anodic electro-fermentation of sugars into ethanol and its optimization with a combination of strain and process improvements. The SCMEC was driven by a bioanode of an alcohol-tolerant strain of *G. sulfurreducens* suitable for operation in ethanol fermentations [21]. Additionally, we genetically engineered the exoelectrogen to prevent the use of cathodic H_2 as an electron donor and used adaptive evolution to remove metabolic constraints that prevented the efficient oxidation of lactate in the parental strain of *G. sulfurreducens* [22]. We demonstrated the efficiency of the improved bioanode during the electro-fermentation of the cellulosic sugar unit, cellobiose, by the fermentative bacterium *C. uda* in a SCMEC. The anodic electro-fermentation prevented pH imbalances and stimulated ethanol titers, yields and productivity. As a result, energy recoveries as ethanol fuel were significantly increased during the electro-fermentation, with a small additional energy recovery as cathodic H_2. The advantages of this electro-fermentation platform to harness energy from complex organic wastes and facilitate the recovery of products from the fermentation broth are discussed.

2. Experimental

2.1. Bacterial strains and culture conditions

The ancestral strain of *G. sulfurreducens* used in this study was

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