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Combining microbial cultures for efficient production of electricity from butyrate in a microbial electrochemical cell

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HIGHLIGHTS

- Two complementary mixed cultures were identified and combined in an MXC.
- The combined culture was capable of producing current from butyrate, via acetate.
- The combined culture rivals enriched cultures in current density and efficiency.
- We identified the organisms responsible for both acetate and current production.
- The right microbial partners can perform complex processes in MXCs.

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ABSTRACT

Butyrate is an important product of anaerobic fermentation; however, it is not directly used by characterized strains of the highly efficient anode respiring bacteria (ARB) *Geobacter sulfurreducens* in microbial electrochemical cells. By combining a butyrate-oxidizing community with a *Geobacter* rich culture, we generated a microbial community which outperformed many naturally derived communities found in the literature for current production from butyrate and rivaled the highest performing natural cultures in terms of current density (~11 A/m^2) and Coulombic efficiency (~70%). Microbial community analyses support the shift in the microbial community from one lacking efficient ARB in the marine hydrothermal vent community to a community consisting of ~80% *Geobacter* in the anode biofilm. This demonstrates the successful production and adaptation of a novel microbial culture for generating electrical current from butyrate with high current density and high Coulombic efficiency, by combining two mixed microbial cultures containing complementing biochemical pathways.

1. Introduction[1]

Microbial electrochemical cells (MXCs) use anode respiring bacteria (ARB) as catalysts to extract electrons from reduced organic compounds and transfer them to an anode, providing electrical current for various applications. MXC technology has been proposed as a complimenting process for current wastewater treatment techniques (Oh et al., 2010). In this setting, MXCs reclaim a small amount of energy from wastewaters while simultaneously decreasing energy input into the treatment process. In order to accomplish this, ARB either produce current directly from the substrates supplied (Kim et al., 1999) or rely on other organisms to hydrolyze and ferment complex substrates prior to current production (Kiely et al., 2011; Parameswaran et al., 2009; Torres et al., 2007).

Following fermentation of complex compounds and wastewater streams, significant amounts of butyrate often remain (Agler et al., 2011), containing up to 45% of the remaining electrons (Fang et al., 2002). Unlike acetate, butyrate is not used as an electron donor by *Geobacter sulfurreducens* (Caccavo et al., 1994), one of the ARB found most often in MXC systems and linked to high current

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[1] *Abbreviation:* MHV, marine hydrothermal vent.

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densities. Under low partial pressures of hydrogen, such as in the presence of methanogens, butyrate fermentation to acetate and hydrogen becomes thermodynamically favorable (Kleerebezem and Stams, 2000). This is also important because high concentrations of volatile fatty acids, including butyrate, inhibit the complete anaerobic degradation of more complex compounds (Siegert and Banks, 2005). MXCs which consume fatty acids, beyond just acetate, therefore stand to prosper by additionally increasing the degradation of more complex substrates.

Previous studies have demonstrated that mixed cultures from the environment and domestic wastewaters are capable of producing electricity from butyrate in MXCs (Freguia et al., 2010; Liu et al., 2005; Min and Logan, 2004; Teng et al., 2010; Torres et al., 2007; Zhang et al., 2011). The fraction of substrate removed and converted to current, or the Coulombic efficiency (CE), with butyrate as electron donor ranges from 8% to 67%, while current densities varied from 0.16 A/m^2 to 0.77 A/m^2 (Freguia et al., 2010; Liu et al., 2005; Torres et al., 2007). It is important to note that these current densities generated are often well below those obtained with ARB fed acetate as the sole electron donor (~10 A/m^2). In many cases variations in current density can be related to the design of the reactors used, including factors such as cathodic potential losses and ohmic resistance, or how the system is operated (fixed potential vs floating potential); however, the microbial community used to convert substrates to current remains a key factor regardless of the reactor design. It appears that simply relying on naturally derived inocula or even mixed communities developed for other purposes is not the ideal method for obtaining efficient microbial cultures for converting complex compounds to electricity in MXCs.

The development of co-cultures with the intent of generating increased current from particular substrates is an area that has been recently raising attention; so far research has focused on combining pure cultures with defined activities. Known ARB have been paired with different organisms in order to generate current from glucose (Rosenbaum et al., 2011), cellulose (Ren et al., 2007) and corn stover (Speers and Reguera, 2012). Read et al. (2010) also demonstrated that when known ARB were paired with *Clostridium acetobutylicum* and fed a mixture of acetate and lactate, current production decreased compared to the ARB alone, although a detailed explanation was not provided. It is quite clear that further work is needed in order to understand both the possibilities and limits of generating novel microbial cultures for electricity and H_2 production in MXCs. Such work should focus on the mechanisms which enable syntrophic interactions to form between microbial populations and improve CE. Given the complexity of wastewaters, we not only require efficient pure cultures, but also robust microbial communities that are suitable for real world, large scale systems.

In this work, we inoculated butyrate fed H-type microbial electrolysis cells (MECs) with two different but complementary mixed microbial cultures. One inoculum came from a marine hydrothermal vent (MHV) and had the capacity to ferment butyrate to acetate during batch culturing. The other inoculum was from a wastewater treatment plant but had been previously enriched in an MEC resulting in a large proportion of *Geobacter* in the population and was able to generate current through the consumption of acetate. We hypothesized that by combining these complementing cultures we could produce an efficient combined culture able to ferment butyrate and produce high current densities in an MEC.

2. Methods

2.1. Microbial cultures for inoculating MECs

For these experiments, marine hydrothermal vent sediment samples were obtained from shallow hydrothermal vents (depth 10 m) located in Punta Mita, Nayarit, Mexico (Mexican Pacific Ocean coast) and registering an average temperature of 85 °C (Guerrero-Barajas and García-Peña, 2010). Pyrite (FeS) is present at this location suggesting the biological formation of sulfide produced by the reduction of sea water sulfate and its combination with the Fe2+ derived from iron oxides and the corresponding reactions with organic matter. Hydrothermal vents are a natural source of microbial diversity responsible for biogeochemical cycling in regular and extreme conditions depending on the geology of the sites. The conditions established in these sites favor the presence of sulfate reducing bacteria (SRB), which promote the production of sulfides commonly found in the deposits of these hydrothermal regions. Previous work documented that these sediments harbored a microbial consortium capable of fermenting butyrate to acetate under sulfate reducing conditions (Guerrero-Barajas and García-Peña, 2010). The samples were stored in the dark and at 4 °C prior to use and contained a volatile suspended solids (VSS) content of 0.037 gVSS/g wet sediment as reported previously (Guerrero-Barajas and García-Peña, 2010). The pH of the sample was 8.91. The ARB inoculum was effluent from an acetate fed MEC seed reactor inoculated with anaerobic sludge from the Mesa Wastewater Treatment Plant, Mesa, AZ. The ARB biofilms of previous MECs started with samples from this waste water treatment plant have routinely contained a significant population of *G. sulfurreducens* cells (Parameswaran et al., 2009; Torres et al., 2009). The MEC used for inoculum was producing steady current (~9 A/m^2) under a poised anode (−300 mV vs Ag/AgCl) at the time of inoculum collection.

2.2. Mineral media

Batch cultures for the enrichment of sediments and MEC systems contained mineral media prepared as reported by Parameswaran et al. (2009). The media contained 3 g/L Na_2HPO_4, 0.512 g/L KH_2PO_4, 0.41 g/L NH_4Cl, 2 g/L NaCl, 10 mL of a trace mineral solution, 1 mL of a 4 g/L $FeCl_2$ solution and 0.5 mL of a 37.2 g/L $Na_2SO_4{\cdot}9H_2O$ solution. The composition of the trace mineral solution was also previously reported by Parameswaran et al. (2009). Sodium acetate and sodium butyrate (at 14 mM) were used as substrates for the different experiments. The enrichment cultures also contained 15 mM sulfate as the electron acceptor.

2.3. Enrichment of sulfate reducing fatty acid consumers

Batch experiments verified the capacity of the MHV microbial population to use either acetate as electron donor with sulfate as the electron acceptor and to ferment butyrate to acetate. Batch experiments were performed in serum bottles with 160 ml total volume; 5 g of wet sediments and 100 mL of media at an initial pH of 7.6 was added to each bottle. After sealing the bottles with Teflon lined rubber septum and aluminum crimps, N_2 sparging of the headspace for 15 min ensured an anaerobic atmosphere. Incubation of the bottles took place in a shaker at 37 °C. Liquid and gas phase samples were taken periodically for analysis.

2.4. MEC setup

MXCs were operated in MEC mode in order to better retain anaerobic conditions inside and to keep the anode constantly polarized. A multi-potentiostat (Bio-Logic VMP3) monitored current production and poised the anode at −200 mV vs Ag/AgCl using an Ag/AgCl reference electrode (BASi Instruments). This potential was chosen to avoid any limitations to already characterized, efficient ARB (*Geobacter*) for growth on the anode, while encouraging the selection of any other novel ARB from the sediments by providing a non-limiting anode potential. The MECs were H-types,