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

Joseph F. Miceli III^a, Ines Garcia-Peña^b, Prathap Parameswaran^a, César I. Torres^{a,c}, and Rosa Krajmalnik-Brown^{a,d,*}

^a Swette Center for Environmental Biotechnology, Biodesign Institute, Arizona State University, P.O. Box 875701, Tempe, AZ 85287-5701, USA

^b Bioprocesses Department, Unidad Profesional Interdisciplinaria de Biotecnología, IPN P.O. Box 07340, Mexico City, Mexico

^c Chemical Engineering Program, School for Engineering of Matter, Transport and Energy, Arizona State University, Tempe, AZ, USA

^d School of Sustainable Engineering and the Built Environment, Arizona State University, Tempe, AZ, USA

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 ($\sim 11 \text{ A/m}^2$) and Coulombic efficiency ($\sim 70\%$). 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 $\sim 80\%$ *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.

Keywords

Short chain fatty acids; Fermentation; *Geobacter*; Syntrophy; Microbial fuel cell

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* Corresponding author at: Swette Center for Environmental Biotechnology, Biodesign Institute, Arizona State University, P.O. Box 875701, Tempe, AZ 85287-5701, USA. Tel.: +1 480 727 7574; fax: +1 480 727 0889. Dr.Rosy@asu.edu (R. Krajmalnik-Brown).. cit@asu.edu (C.I. Torres)

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2014.06.090>.

1. Introduction¹

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 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/m² to 0.77 A/m² (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/m²). 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),

¹Abbreviation: MHV, marine hydrothermal vent.

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 H₂ 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/m²) 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 $\text{Na}_2\text{SO}_4 \cdot 9\text{H}_2\text{O}$ 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, consisting of two graphite rod electrodes (electrode 1: 6.55 cm $\times$ 0.3 cm; electrode 2: 6.05 cm $\times$ 0.4 cm; total surface area ~ 14.1 cm²; graphitstore.com) for the anodes and two (both 6.55 cm $\times$ 0.3 cm; total surface area ~ 12.6 cm²) for the cathodes. The two chambers were separated by an anion exchange membrane (AMI-7001, Membranes International). The cathode chamber contained distilled water adjusted to pH 12 with NaOH and the anode chamber with the same medium described above (~ 360 mL). Fig. S1 in the supplementary information is a schematic of the MEC setup.

After initial set up, the anode chamber was sparged with pure N_2 gas in order to obtain anaerobic conditions. The anode and cathode chambers were stirred with magnetic stir bars at 150 rpm. Four MECs were setup in this manner and kept in an incubator at 37 °C. The control experiment (MEC 1) consisted of 25 mM butyrate in the media and 5 mL of ARB inoculum. Two MECs were inoculated with 20 g of sediment from the marine hydrothermal vent, MEC 2 and MEC 3. 25 mM butyrate as electron donor was provided to MEC 2 and 25 mM acetate to MEC 3. Both MEC 2 and 3 were performed in duplicate. Finally, MEC 4 contained 25 mM butyrate, 20 g of sediment, and 5 mL of ARB inoculum. MEC 4 was initially run in batch mode, but was eventually set up in continuous flow mode at a hydraulic retention time of 1 day.

2.5. Substrate and metabolite analysis

Liquid samples (1 mL) from the suspension of the MECs and the batch microbial cultures were filtered with 0.22 μ m syringe filters (Pall Life Sciences Cat 4455) and analyzed using high performance liquid chromatography (performed on a HPLC Model LC-20AT, Shimadzu) to quantify volatile fatty acids and alcohols, including acetate and butyrate. The samples were analyzed on a HPX-87H (Bio-Rad Laboratories) column at 50 °C with sulfuric acid (2.5 mM) as the eluent at 0.6 mL/min in order to separate the components and the effluent was analyzed using a photo-diode array (at 210 nm) over 35 min. Gas samples (200 μ L volume) from the cultures and MECs were taken using a gas-tight syringe (SGE 500 μ L, Switzerland) and analyzed by gas chromatography (GC) for gaseous products of microbial activity, specifically CO₂, CH₄ and H₂. The samples were analyzed on a Shimadzu GC 2010, using a packed column (ShinCarbon ST 100/120 mesh, Restek Corporation, Bellefonte, PA) for separation and a thermal conductivity detector. The carrier gas was N₂ fed at 10 mL/min at 5.4 atm. The injection loop was set to 110 °C, the column to 140 °C, and the detector to 160 °C. A standard curve was made for calibration using analytical grade H₂, CH₄, and CO₂.

2.6. Community analyses

Biomass from the marine hydrothermal vent sediment as well as the biofilm and suspension portions of MECs 1 and 4 at the end of each experiment were used for community analysis. DNA was extracted from the biomass using the MoBio Powersoil DNA Extraction kit according to the manufacturer's directions. A clone library was performed on the DNA extracted from MEC 1 using primers targeted to the 16S rRNA gene as previously described (Torres et al., 2009). 52 clones were picked and sequenced from the sediment, 43 from the biofilm, and 23 clones from the suspension.

DNA extracted from the MHV sediment and the biofilm and suspension of MEC 4 was sent to Research and Testing Laboratory (Lubbock, TX) for 454 pyrosequencing as described in Garcia-Peña et al., 2011. The gene target was the 16s rRNA gene of general bacteria using the commercially available Blue primers (104F-530R) targeting the V2 and V3 regions of the 16s rDNA. Sequences were analyzed using the mothur software (www.mothur.org) as described in Garcia-Peña et al., 2011. Single sequences were removed and the number of sequences normalized in all sets to that of the smallest set: 5973 sequences. For classification, a bootstrap cutoff of 50% was used as recommended by RDP when using sequences below 250 bp in length.

Quantitative real-time PCR was used to quantify methanogens in DNA extracted from the MHV sediment as well as the biofilm and suspension from MEC 4 using TaqMan probes. The probes targeted general *Archaea* (primers Arc787F, Arc1059R, Taqman probe Arc915F), the orders *Methanobacteriales* (primers MBT857F, MBT1196R, Taqman probe MBT929F) and *Methanomicrobiales* (primers MMB282F, MMB832R, Taqman probe MMB749F), and the families *Methanosarcinaceae* (primers Msc 38F, Msc828R, Taqman probe Msc492F) and *Methanosaetaceae* (primers Mst702F, Mst862R, Taqman probe Mst753F) as previously described (Yu et al., 2005). Reactions were performed in 10 μ L volumes on 96 well plates. Each reaction mixture contained 4.5 μ L of Real Master Mix (5'

Prime, Cat #2900237), 0.03 μL of Taqman Probe (final concentration 300 nM), 0.5 μL each of forward and reverse primers (final concentration 5 μM) and 4 μL of template DNA at ~ 10 ng/ μL . Taqman probes were labeled with FAM and the reactions were performed and monitored using a Realplex Mastercycler ep gradient S (Eppendorf). *Archaea*, *Methanobacteriales*, *Methanomicrobiales*, and *Methanosaetaceae* assays were initiated at 93 $^{\circ}\text{C}$ for 30 s followed by 45 cycles of melting (93 $^{\circ}\text{C}$ for 10 s) and binding/elongation (63 $^{\circ}\text{C}$ for 30 s). For *Methanosarcinaceae*, binding/elongation was performed at 60 $^{\circ}\text{C}$.

2.7. Substrate spike experiments

In order to investigate if butyrate was directly consumed by the biofilm formed or if there was a need for initial fermentation of butyrate to acetate by the co-mixed culture prior to utilization by ARB, spike experiments were performed on MEC 4 in batch mode after it had a well-developed biofilm, grown under continuous flow operation. The current in a substrate-depleted MEC was first allowed to decrease below <0.5 A/ m^2 . Then, either butyrate or acetate was introduced to the MEC using a syringe. Butyrate was introduced at 1 M (5 mL) to a final concentration of ~ 14 mM. Acetate was introduced at 1 M (5 mL) to a final concentration of ~ 14.2 mM. The instantaneous change in current density after each substrate spike was recorded.

3. Results and discussion

3.1. Acetate and butyrate consumption in batch experiments

In order to enrich the microbial population of the hydrothermal sediments for either acetate or butyrate consumers, we inoculated sediments in two batch cultures using either acetate or butyrate as electron donor and sulfate as electron acceptor (data available in the supplementary information [SI], Fig. S2). Acetate was used as a control to show that the sediments were viable and that the culture technique was appropriate to grow anaerobic sulfate reducers, which include relatives of *Geobacter*, regardless of successful butyrate fermentation. In the acetate fed cultures, around 70% of the total acetate supplied was consumed during the first four days of cultivation; complete acetate degradation occurred after 11 days. In the butyrate fed cultures, butyrate was initially transformed to acetate by the microbial population starting at day 4 and the complete removal of both substrates was observed after 11 days. Detection of CO_2 gas in the headspace indicates that some mineralization of acetate and butyrate occurred. This data suggested the possible use of the MHV sediments in MEC systems using either acetate or butyrate as electron donors.

3.2. Current generation by individual microbial cultures

Two separate mixed culture MECs (MECs 1 and 2) proved unable to produce high current densities from butyrate. When we placed the inocula from a highly enriched, current producing, acetate fed MEC in a new MEC with butyrate as the sole electron donor (MEC 1), very little current was produced (<0.1 A/ m^2 , see Fig. 1, panel A). Our acetate-fed MEC inoculum consisted mostly of *G. sulfurreducens* (Torres et al., 2009), an ARB known to consume acetate but not butyrate (Caccavo et al., 1994). Without organisms to ferment butyrate to acetate, the *Geobacter* rich culture could not produce current from butyrate. Both MHV sediment culture only MECs (MECs 2 and 3) also showed only minor electrogenic

activity, producing current densities of $\sim 0.2 \text{ A/m}^2$ (butyrate fed MEC 2 shown in Fig. 1, panel B; acetate fed MEC 3 data not shown). Fig. 2 shows the distribution of electrons in MEC 2 where butyrate was clearly fermented to acetate. In spite of this, only a very small amount of the electrons provided as butyrate were ultimately channeled into current, indicating either a lack of acetate consuming ARB or unfavorable conditions for current production. MEC 3's failure to generate more than $\sim 0.2 \text{ A/m}^2$ further confirmed that the mixed culture from the MHV sediments lacked acetate consuming ARB.

We analyzed the microbial community of the MHV sediment as well as the suspension and the small biofilm attached to the electrode of MEC 2 using a clone library (see SI, Fig. S4). The organisms detected were mainly fermenters along with some organisms known for only weak interactions with the anode. No *G. Sulfurreducens* sequences were detected.

3.3. Current generation by combining the MHV and *Geobacter* rich ARB cultures in a butyrate fed MEC

Next, we amended the MHV sediment culture with the *Geobacter* containing culture in an MEC fed with butyrate (MEC 4). Once combined together, this co-mixed culture fermented butyrate and successfully produced electricity. Current densities (Fig. 1, panel C) were similar to those found previously with *Geobacter* cultures fed on only acetate ($\sim 10 \text{ A/m}^2$) in other fixed potential systems. Data from volatile fatty acid analysis indicates that the electricity was produced through intermediate acetate production and consumption (Fig. 3). CE for this culture was 70% by the end of the experiment. Methane was detected by GC and hydrogenotrophic methanogens (*Methanobacteriales*) were detected using qPCR (see SI, Fig. S3). The missing electrons in Fig. 3 were likely diverted to methane and biomass, although neither sinks were quantified. The high recovery of electrons in the MHV culture alone as products (Fig. 2) was likely due to greater biomass being present, resulting in a very small loss of electrons to biomass production.

The ARB and butyrate fermenting organisms appear to be largely physically separated in MEC 4, as observed using pyrosequencing. A summary of the community analysis results at the class level are shown in Fig. 4 along with community data from the ARB inoculum published previously (Torres et al., 2009). *Deltaproteobacteria* (the class that includes the known ARB *Geobacter*) dominate the microbial culture in the current producing biofilm, similar to the current producing biofilm community used as ARB inoculum. Evaluating these data together with the high current density obtained and the presence of acetate in the system, we therefore conclude that *Geobacter* are the main ARB generating current.

The suspension appears to demonstrate influences from both the sediment and the biofilm communities: there is a high proportion of *Gammaproteobacteria*, similar to the sediment, and some *Clostridia*, *Betaproteobacteria* and *Epsilonproteobacteria* which are seen in the biofilm and suspension but not the original sediment. *Clostridia* have been recorded previously in a MXC fed with butyrate and inoculated with anaerobic sludge (Chae et al., 2009) while *Gammaproteobacteria* are known fermenters and have been linked to the production of current from glucose (Chung and Okabe, 2009) and acetate (Pham et al., 2003). Butyrate is fermented to acetate by a mixture of mainly *Gammaproteobacteria* and

Clostridia. The large proportion of *Deltaproteobacteria* sequences found in the suspension likely resulted from anode biofilm detachment.

This physical separation between communities performing different functions has important implications, both positive and negative, for the design of MXC systems. Suspended and biofilm communities can be subjected to different retention times for the control of biomass production. Unfortunately, suspended communities will also be more susceptible than biofilm communities to washout at higher flow rates.

3.4. Current response to acetate and butyrate spikes in MECs

We performed spike experiments on MEC 4 to better understand whether current production came from direct oxidation of butyrate or through intermediate acetate fermentation in our butyrate fed MECs. Organic acid analysis indicates that the consortium fermented butyrate to acetate when butyrate was used as a substrate for electricity production by the ARB. Fig. 5, panel A, shows the lagging response of the anode biofilm upon addition of butyrate, on the order of hours, in comparison to a much quicker response to the addition of acetate, on the order of minutes. Fig. 5, panel B, shows the decrease in butyrate observed over time, with only a minor and temporary increase in acetate concentration. Acetate levels in the bulk liquid (~7 mM) do not reach those often associated with the high levels of current density found here. Given that acetate is a known substrate for ARB biofilms (Caccavo et al., 1994; Freguia et al., 2010; Kiely et al., 2011; Torres et al., 2009), the lag in current response to substrate addition and the presence of acetate in the media indicate that the consortium fermented butyrate to acetate prior to acetate oxidation at the anode for the production of current.

4. Conclusions

This work demonstrates the capacity to amend highly efficient ARB cultures with other microbial cultures displaying interesting metabolic capabilities in order to build metabolic pathways for the utilization of complex feeds in MXCs. This enhanced co-culture rivals and in some cases surpasses those derived naturally or through enrichment by producing high current densities (~10 A/m²) and high CE (~70%). By assembling microbial communities containing the best organisms for each desired metabolic step we can quickly, easily, and reliably produce mixed communities to perform desired applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Agler MT, Wrenn BA, Zinder SH, Angenent LT. Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. *Trends Biotechnol.* 2011; 29:70–78. [PubMed: 21190748]
- Caccavo F, Lonergan DJ, Lovley DR, Davis M, Stolz JF, McInerney MJ. *Geobacter sulfurreducens* sp. nov., a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. *Appl. Environ. Microbiol.* 1994; 60:3752–3759. [PubMed: 7527204]
- Chae K-J, Choi M-J, Lee J-W, Kim K-Y, Kim IS. Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells. *Bioresour. Technol.* 2009; 100:3518–3525. [PubMed: 19345574]
- Chung K, Okabe S. Continuous power generation and microbial community structure of the anode biofilms in a three-stage microbial fuel cell system. *Appl. Microbiol. Biotechnol.* 2009; 83:965–977. [PubMed: 19404637]
- Fang HHHP, Liu H, Zhang T. Characterization of a hydrogen-producing granular sludge. *Biotechnol. Bioeng.* 2002; 78:44–52. [PubMed: 11857280]
- Freguia S, Teh EH, Boon N, Leung KM, Keller J, Rabaey K. Microbial fuel cells operating on mixed fatty acids. *Bioresour. Technol.* 2010; 101:1233–1238. [PubMed: 19854639]
- García-Peña EI, Parameswaran P, Kang DW, Canul-Chan M, Krajmalnik-Brown R. Anaerobic digestion and co-digestion processes of vegetable and fruit residues: process and microbial ecology. *Bioresour. Technol.* 2011; 102:9447–9455. [PubMed: 21865034]
- Guerrero-Barajas C, García-Peña EI. Evaluation of enrichments of sulfate reducing bacteria from pristine hydrothermal vents sediments as potential inoculum for reducing trichloroethylene. *World J. Microbiol. Biotechnol.* 2010; 26:21–32.
- Kiely PD, Regan JM, Logan BE. The electric picnic: synergistic requirements for exoelectrogenic microbial communities. *Curr. Opin. Biotechnol.* 2011; 22:378–385. [PubMed: 21441020]
- Kim B, Kim H, Hyun M, Park D. Direct electrode reaction of Fe(III)-reducing bacterium, *Shewanella putrefaciens*. *J. Microbiol. Biotechnol.* 1999; 9:127–131.
- Kleerebezem R, Stams AJM. Kinetics of syntrophic cultures: a theoretical treatise on butyrate fermentation. *Biotechnol. Bioeng.* 2000; 67:529–543. [PubMed: 10649228]
- Liu H, Cheng S, Logan BE. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. *Environ. Sci. Technol.* 2005; 39:658–662. [PubMed: 15707069]
- Min B, Logan BE. Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. *Environ. Sci. Technol.* 2004; 38:5809–5814. [PubMed: 15575304]
- Oh ST, Kim JR, Premier GC, Lee TH, Kim C, Sloan WT. Sustainable wastewater treatment: how might microbial fuel cells contribute. *Biotechnol. Adv.* 2010; 28:871–881. [PubMed: 20688144]
- Parameswaran P, Torres CI, Lee H-S, Krajmalnik-Brown R, Rittmann BE. Syntrophic interactions among anode respiring bacteria (ARB) and Non-ARB in a biofilm anode: electron balances. *Biotechnol. Bioeng.* 2009; 103:513–523. [PubMed: 19191353]
- Pham CA, Jung SJ, Phung NT, Lee J, Chang IS, Kim BH, Yi H, Chun J. A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Aeromonas hydrophila*, isolated from a microbial fuel cell. *FEMS Microbiol. Lett.* 2003; 223:129–134. [PubMed: 12799011]
- Read ST, Dutta P, Bond PL, Keller J, Rabaey K. Initial development and structure of biofilms on microbial fuel cell anodes. *BMC Microbiol.* 2010; 10:98–108. [PubMed: 20356407]
- Ren Z, Ward TE, Regan JM. Electricity production from cellulose in a microbial fuel cell using a defined binary culture. *Environ. Sci. Technol.* 2007; 41:4781–4786. [PubMed: 17695929]
- Rosenbaum MA, Bar HY, Beg QK, Segrè D, Booth J, Cotta MA, Angenent LT. *Shewanella oneidensis* in a lactate-fed pure-culture and a glucose-fed co-culture with *Lactococcus lactis* with an electrode as electron acceptor. *Bioresour. Technol.* 2011; 102:2623–2628. [PubMed: 21036604]
- Siebert I, Banks C. The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. *Process Biochem.* 2005; 40:3412–3418.

- Speers AM, Reguera G. Consolidated bioprocessing of AFEX-pretreated corn stover to ethanol and hydrogen in a microbial electrolysis cell. *Environ. Sci. Technol.* 2012; 46:7875–7881. [PubMed: 22697183]
- Teng S-X, Tong Z-H, Li W-W, Wang S-G, Sheng G-P, Shi X-Y, Liu X-W, Yu H-Q. Electricity generation from mixed volatile fatty acids using microbial fuel cells. *Appl. Microbiol. Biotechnol.* 2010; 87:2365–2372. [PubMed: 20607228]
- Torres CI, Krajmalnik-Brown R, Parameswaran P, Marcus AK, Wanger G, Gorby YA, Rittmann BE. Selecting anode-respiring bacteria based on anode potential: phylogenetic, electrochemical, and microscopic characterization. *Environ. Sci. Technol.* 2009; 43:9519–9524. [PubMed: 20000550]
- Torres CI, Marcus AK, Rittmann BE. Kinetics of consumption of fermentation products by anode-respiring bacteria. *Appl. Microbiol. Biotechnol.* 2007; 77:689–697. [PubMed: 17909786]
- Yu Y, Lee C, Kim J, Hwang S. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnol. Bioeng.* 2005; 89:670–679. [PubMed: 15696537]
- Zhang Y, Min B, Huang L, Angelidaki I. Electricity generation and microbial community response to substrate changes in microbial fuel cell. *Bioresour. Technol.* 2011; 102:1166–1173. [PubMed: 20952193]

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.

Fig. 1. Current density from three MECs: (A) butyrate fed *Geobacter* culture (MEC 1), (B) butyrate fed MHV culture (MEC 2), and (C) butyrate fed, MHV-*Geobacter* co-mixed culture (MEC 4).

Fig. 2.

Electron balance for MHV sediment culture in an MEC, fed with butyrate.

Fig. 3.

Electron balance for a butyrate fed MEC inoculated with a culture from a marine hydrothermal vent capable of butyrate fermentation and a *Geobacter* rich culture enriched from anaerobic wastewater sludge (MEC 4).

Fig. 4.

Microbial community analysis at the class level for the hydrothermal vent sediment, the butyrate fed MHV-*Geobacter* co-mixed culture MEC 4 Suspension, the same MEC's Biofilm, and the ARB inoculum used (previously published in Torres et al., 2009), highlighted in grey as the inoculum was analyzed at an earlier time point.

Fig 5.

Co-mixed culture: (A) current response to butyrate and acetate spikes and (B) butyrate and acetate concentrations during butyrate spike.