



## Long-term cathode performance and the microbial communities that develop in microbial fuel cells fed different fermentation endproducts

Patrick D. Kiely, Geoffrey Rader, John M. Regan, Bruce E. Logan \*

Department of Civil and Environmental Engineering, 131 Sackett Building, The Pennsylvania State University, University Park, PA 16802, USA

### ARTICLE INFO

#### Article history:

Received 17 March 2010  
Received in revised form 4 May 2010  
Accepted 6 May 2010  
Available online 31 May 2010

#### Keywords:

Microbial community  
Microbial fuel cells  
Cathodes  
Biocathodes

### ABSTRACT

To better understand how cathode performance and substrates affected communities that evolved in these reactors over long periods of time, microbial fuel cells were operated for more than 1 year with individual endproducts of lignocellulose fermentation (acetic acid, formic acid, lactic acid, succinic acid, or ethanol). Large variations in reactor performance were primarily due to the specific substrates, with power densities ranging from  $835 \pm 21$  to  $62 \pm 1$  mW/m<sup>3</sup>. Cathodes performance degraded over time, as shown by an increase in power of up to 26% when the cathode biofilm was removed, and 118% using new cathodes. Communities that developed on the anodes included exoelectrogenic families, such as *Rhodobacteraceae*, *Geobacteraceae*, and *Peptococcaceae*, with the *Deltaproteobacteria* dominating most reactors. *Pelobacter propionicus* was the predominant member in reactors fed acetic acid, and it was abundant in several other MFCs. These results provide valuable insights into the effects of long-term MFC operation on reactor performance.

© 2010 Elsevier Ltd. All rights reserved.

### 1. Introduction

Microbial fuel cells (MFCs) are systems that generate electrical current through the microbial oxidation of fuels and associated reduction of an anode electrode. Fuels used in MFCs range from simple molecules to complex waste organics (Lovley, 2008). Substrates of particular interest are the soluble byproducts of dark fermentation. Cellulose and sugars can only be partially oxidized in a dark-fermentation process. One mole of glucose has enough electrons to produce 12 mol of hydrogen, but the maximum theoretical yield by dark fermentation is 4 mol of hydrogen (33%) when two moles of acetate are produced as the only byproduct. In practice, hydrogen yields are much lower (17–23%) (Logan, 2004) due to the build-up of a variety of other fermentative byproducts, including volatile fatty acids such as lactic, formic, butyric, propionic, and succinic acids, and alcohols and solvents (Lalauette et al., 2009; Ren et al., 2009). The ability to harness these byproducts and convert them to electrical current in an MFC or hydrogen in a microbial electrolysis cell (MEC) is therefore an attractive method for recovering additional energy (Lalauette et al., 2009).

A significant effort has been made to improve MFC performance by optimizing the reactor architecture (Cheng and Logan, 2007; Watanabe, 2008), with less attention to the long-term performance of electrodes, particularly with respect to the cathode. Changes in power density and Coulombic efficiencies (CEs) were found for

reactors operated over 60 days (30 cycles) (Yang et al., 2009; Zhang et al., 2009), with a pattern over time of decreased power densities due to development of a biofilm on the cathode (Cheng et al., 2006). While formation of a cathode biofilm reduced power generation, it also increased the CEs, i.e. the percent of substrate electrons recovered as current, due to a reduction of oxygen intrusion into the anode chamber. The type of substrates used in the MFC is also important relative to long-term performance. Cathode catalysts become inactivated to an extent dependent on the specific substrate, with a substantial reduction in efficiency found for formate and ethanol but relatively little effect observed for acetate relative to organic oxidation rates (Harnisch et al., 2009). The long-term effect of different substrates on cathode catalytic performance relative to the biofilm has not been previously examined.

The long-term evolution of the microbial communities in anode biofilms is also important relative to MFC performance. MFCs have been found to vary widely in diversity, containing only a few dominant microorganisms in some cases (Kim et al., 2007) or very diverse populations in other studies (Chae et al., 2009). In one study that used denaturing gradient gel electrophoresis (DGGE) screening and sequencing of amplified 16S rRNA gene fragments, there was little diversity found for two-chamber MFCs fed three different substrates (acetate, lactate, and glucose), with communities dominated by sequences closely affiliated with *Geobacter sulfurreducens* (99% similarity) (Jung and Regan, 2007). In contrast, a substantially more diverse array of microbes was found (Chae et al., 2009) for MFCs individually fed acetate, butyrate, propionate, and glucose. Although *Deltaproteobacteria* were identified in all

\* Corresponding author. Tel.: +1 814 863 7908; fax: +1 814-863-7304.  
E-mail address: [blogan@psu.edu](mailto:blogan@psu.edu) (B.E. Logan).

reactors, they comprised a relatively small proportion of the total microbial consortia present on the anode (Chae et al., 2009). These different findings relative to microbial community diversity could be due to a number of factors, including differences in reactor architecture, operation time, inoculum, as well as the technique used to characterize the community. Clone libraries are now increasingly used to characterize microbial populations as they offer a more quantitative profile of the microbial community structure (Deng et al., 2008). Microbial communities in MFCs likely evolve over time, but often these communities are characterized after only a few weeks to months of operation (Ishii et al., 2008), although in some cases longer operation times of 1 year have been examined (Chae et al., 2009). The selective pressure of the MFC environment can result in changes in microbial populations that alter power output, internal resistance, and that can decrease mass transfer limitations (Aelterman et al., 2006). Thus, it may be that long periods of time (months to a year) are needed to study microbial community dynamics in MFCs.

In order to better understand how the electrode conditions evolve in reactors operated for long periods of time, we examined how MFCs that had been operating for more than 1 year change with five different substrates (acetic acid, formic acid, lactic acid, succinic acid, or ethanol). These substrates were chosen because they were the main endproducts produced from lignocellulose fermentation by *Clostridium thermocellum* (Lalaurette et al., 2009). We evaluated reduced cathode performance due to the biofilm by first removing the biofilm and operating the reactor for several cycles. Next, we replaced the cathode with a new cathode to determine to what extent both cathode catalytic activity and the biofilm limited performance. The reactors differed appreciably in terms of voltages produced, so we also examined the microbial communities that evolved on the anodes after this long period of operation using 16S rRNA gene clone libraries.

## 2. Methods

### 2.1. MFC construction

Air–cathode MFCs were constructed from a solid block of Lexan to contain a single cylindrical chamber (28 mL) as previously described (Logan et al., 2007). Anodes were graphite fiber brushes (Logan et al., 2007) 2.5 cm in diameter and 2.5 cm long (PANEX33 160K, ZOLTEK) treated with a high-temperature ammonia gas process (Cheng and Logan, 2007). Cathodes contained 0.5 mg/cm<sup>2</sup> Pt and four diffusion layers on 30 wt.% wet-proofed carbon cloth (7 cm<sup>2</sup>, type B-1B, E-TEK) (Cheng et al., 2006).

### 2.2. MFC operation

The inoculum was effluent from a primary clarifier at the Pennsylvania State University Wastewater Treatment Plant. Reactors were refilled with different substrates in fresh nutrient medium (50 mM phosphate buffered saline (PBS), 1 g/L of relevant electron donor, Wolfe's trace minerals (12.5 ml/L) and minerals (5 ml/L)) (Balch et al., 1979) when the voltage dropped below 20 mV. Single substrates (acetic acid, formic acid, ethanol, lactic acid, succinic acid) were added to individual MFCs at a concentration of 1 g/L (Lalaurette et al., 2009). The biofilm on the cathode was removed by washing gently in DI water. All tests were conducted at ambient temperatures (23 ± 3 °C), with results reported based on tests in triplicate, for duplicate reactors with each substrate.

Voltage ( $E$ ) was measured across an external 1 k $\Omega$  resistor using a data acquisition system (2700; Keithly, United States) to calculate the current ( $I = E/R$ ) and power ( $P = IE$ ), where  $R$  is external resistance. Polarization and power density curves were generated

after the operation of MFCs over a 1 year period. Polarization and power density curve values were attained by MFCs producing a maximum stable voltage by changing the external resistance of the reactors every 20 min from 20 k $\Omega$  down to 50  $\Omega$ . Current and power densities were normalized to the cathode projected surface area ( $A_{\text{cat}}$ ). Internal resistance ( $R_{\text{INT}}$ ) was calculated from the slope of polarization curves. Coulombic efficiency, defined as the percentage of electrons recovered as current in one batch cycle versus the total available electrons from the initial input substrate (e.g., 8 mol e<sup>-</sup>/mol acetate), was calculated as previously described (Lalaurette et al., 2009).

### 2.3. Bacterial community and phylogenetic analysis

DNA extraction from bacteria on the anode biofilm, 16S rRNA gene cloning, and sequencing for community analysis were conducted as previously described (Call et al., 2009). DNA was extracted using a PowerSoil DNA isolation kit (MO BIO Laboratories) from duplicate reactors and pooled. For the mixed communities, a nearly full length 16S rRNA gene fragment of the extracted DNA was amplified by PCR using universal bacterial primers 27F (5'-AGAGTTTGATCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGATCCAGCC-3') (Winker and Woese, 1991). PCR products were purified using QIAquick PCR Purification Kit (Qiagen) and then ligated and cloned using a TOPO TA cloning kit (Invitrogen) according to the manufacturer's instructions. Appropriate colonies were gridded in a 96-well format on LB plates (Amp<sup>r</sup> 50  $\mu$ l/ml). Plasmid extractions were carried out on these colonies using the E-Z 96 Fastfilter<sup>®</sup> Plasmid Kit<sup>®</sup> (Omega Bio-Tek). Plasmid inserts were sequenced with the M13R primer using a DNA sequencer (ABI 3730XL, Applied Biosystems). The nucleotide collection (nr/nt) of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>) was searched using the BLASTn algorithm to analyze the sequences. Mega 4.0.2 (Tamura et al., 2007) was used to align these sequences and generate a phylogenetic tree using the neighbor-joining method with a bootstrap test (500 replicates) of phylogeny. Sequences derived from the analysis of the reactors were deposited in GenBank under accession numbers GU083321–GU083567.

### 2.4. *Geobacter sulfurreducens* PCA and *Pelobacter propionicus* DSM 2378 growth conditions

*G. sulfurreducens* PCA (ATCC 51573) and *Pelobacter propionicus* DSM 2379 were obtained from our laboratory collection and cultured under anaerobic conditions with appropriate electron donor and acceptor as described previously (Call et al., 2009; Schink, 1984). To inoculate the culture, 1 mL of cells plus medium were added to the air–cathode MFCs. The MFC was sparged with anaerobic N<sub>2</sub>/CO<sub>2</sub> (80/20 [vol/vol]) gas prior to inoculation. The MFC medium contained 1 g/L acetic acid in a 50 mM PBS buffer as described above.

### 2.5. Statistical analysis of microbial communities

Rarefaction curves were developed as previously described for each microbial community to determine whether sufficient representative clones had been sequenced (Hughes et al., 2001). 16S rRNA gene fragments of clones representing distinct phylotypes (97% similarity) were plotted against the total number of clones. Sampling coverage (Good's coverage) was calculated as previously described (Good, 1953). Community diversity was determined by calculating the Shannon Diversity Index for each reactor (Bianchi and Bianchi, 1982) as follows,

$$H' = - \sum_{i=1}^S p_i \ln p_i,$$

where  $S$  represents the species richness and  $p_i$  represents the proportion of a phylotype relative to the sum of all phylotypes.

### 3. Results and discussion

#### 3.1. Effect of different substrates on MFC performance

Reactors operated for more than 1 year showed large variations in peak voltages dependent on the specific substrate. Acetic acid-fed reactors produced the highest peak voltage ( $\sim 475$  mV), with lower voltages for succinic ( $\sim 400$  mV) and lactic acids ( $\sim 400$  mV), and ethanol ( $\sim 375$  mV). The formic acid-fed MFC produced a substantially lower voltage of  $\sim 180$  mV (Supplementary data). Typical fed-batch cycles lasted 2–3 days. Voltage was always rapidly produced ( $\sim 20$  min) after the medium was replaced, suggesting direct electron transfer by attached microbes and that mediators were not needed for power production.

Polarization data obtained from the reactors showed that acetic acid-fed reactors could produce the highest maximum power density of  $556 \pm 48$  mW/m<sup>2</sup>, with somewhat lower values for reactors fed lactic acid ( $474 \pm 25$  mW/m<sup>2</sup>) and succinic acid ( $340 \pm 34$  mW/m<sup>2</sup>). Reactors fed ethanol produced approximately half the maximum power density ( $289 \pm 180$  mW/m<sup>2</sup>) of an acetic acid-fed reactor. There was substantially less power produced by the reactor fed formic acid ( $30 \pm 1$  mW/m<sup>2</sup>) (Fig. 1).

After 1 year of operation the water side of the cathode was covered by a thick biofilm (2–3 mm). Removing the biofilm increased the maximum power densities of all reactors except those fed formic acid. The greatest increase occurred in reactors fed acetic acid with values increasing from  $556 \pm 48$  to  $704 \pm 30$  mW/m<sup>2</sup> (Fig. 2). This result indicates that biofilm development impaired power generation, but that the cathode biofilm was not the primary reason for differences in power generation among the MFCs fed different substrates. To determine the extent that deterioration of catalyst was a factor in maximum power densities, the cleaned cathodes were replaced with new cathodes. Polarization curves generated with the new cathodes showed increased, but not equal, maximum power densities with the different substrates (Fig. 2). Reactors fed ethanol showed the greatest improvement in cathode performance over time, with maximum power increasing from  $375 \pm 171$  mW/m<sup>2</sup> prior to cathode replacement to  $820 \pm 24$  mW/m<sup>2</sup> with the new cathodes (Fig. 2). Thus, the catalyst performance was affected differently by the various substrates. The reduction in catalytic ability of cathodes when ethanol is supplied as the MFC substrate is consistent with previous studies (Harnisch et al.,

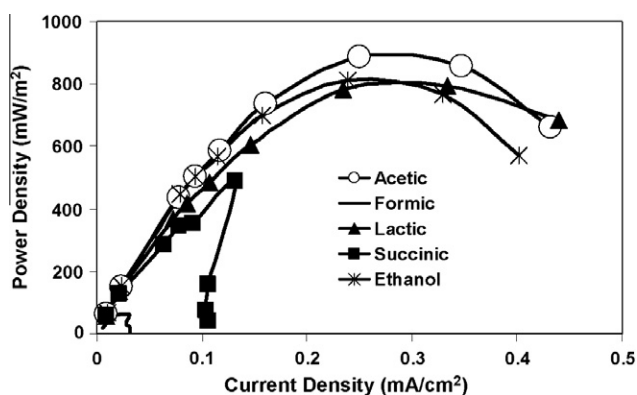


Fig. 1. Power densities produced by MFCs fed different fermentation endproducts.

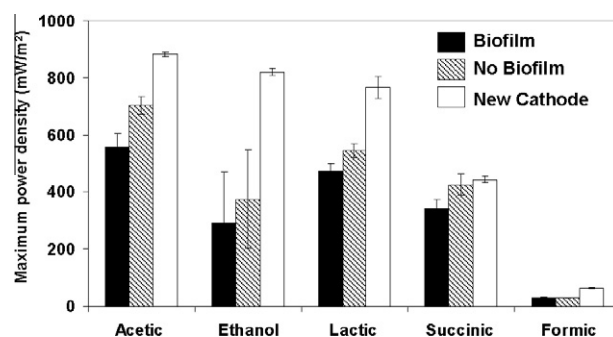


Fig. 2. MFC maximum power densities with cathodes containing biofilm (formed over 1 year of operation), cathodes where the biofilm had been removed (no biofilm), and new cathodes.

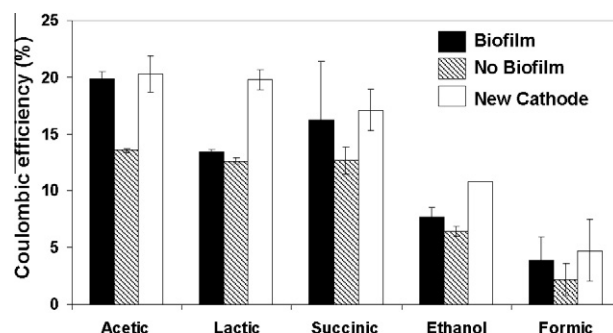


Fig. 3. MFC Coulombic efficiency (CE) with cathodes containing biofilm (formed over 1 year of operation), cathodes where the biofilm had been removed (no biofilm), and new cathodes.

2009). The same study, however, did not predict the reductions in cathode performance when lactic acid or acetic acid is supplied as the MFC substrate (Harnisch et al., 2009).

The CEs for the MFCs containing cathodes with a biofilm were highest for acetic acid ( $19.9 \pm 0.6\%$ ), followed by succinic acid ( $16.2 \pm 5.2\%$ ), lactic acid ( $13.4 \pm 0.3\%$ ), ethanol ( $7.7 \pm 0.8\%$ ), and formic acid ( $3.9 \pm 2.0\%$ ). When the biofilm was removed, the acetic acid MFCs showed the largest decrease in CE from  $19.9 \pm 0.6\%$  to  $13.5 \pm 0.2\%$ . Replacement of the cathode with new cathodes increased CEs, with the largest increase obtained for MFCs fed lactic acid ( $12.6 \pm 0.3 - 19.8 \pm 0.9\%$ ) and ethanol ( $6.4 \pm 0.5 - 10.8 \pm 0.1\%$ ) (Fig. 3). The reduction in CE values after the cathodic biofilm was removed for all reactors is consistent with previous studies (Yang et al., 2009; Zhang et al., 2009). This reduction in CE was accompanied by an increased current density across all reactors. Using a new cathode increases the power density, allowing for a reduction in cycle time and thus an increase in CE due to less loss of substrate to aerobic degradation supported by oxygen diffusion through the cathode (Yang et al., 2009; Zhang et al., 2009).

Internal resistance measurements after cathode replacement showed that reactors fed acetic acid, lactic acid, and ethanol all had similar values, while reactors fed succinic acid were about two times higher and those fed formic acid were an order of magnitude higher (Table 1).

#### 3.2. Community analysis of anodic microbial populations

Community analysis of MFCs fed different substrates showed that there were substantial variations among the dominant microbial consortia in these reactors. Phylogenetic analysis of the clone libraries showed that *Proteobacteria* dominated almost all the an-

**Table 1**

Maximum power density values and internal resistance values attained for reactors operated for 1 year and following cathode replacements.

Substrate	Maximum power density (mW/m <sup>2</sup> )	Internal resistance (Ω)
Formic acid	62 ± 0.03	2769 ± 318
Acetic acid	835 ± 20.5	184 ± 15
Ethanol	820 ± 24	216 ± 8
Lactic acid	739 ± 32.2	159 ± 11
Succinic acid	444 ± 12.5	376 ± 30

ode biofilms. The only exception to this was with the lactic acid-fed reactor, where 52% of the sequenced clones were associated with *Firmicutes* (Table 2). *P. propionicus* was found to be the dominant microbe in MFC reactors fed acetic acid (63% of the anodic community) and lactic acid (39%), and were also a large proportion of clones obtained from reactors fed ethanol (21%) and succinic acid (15%) (Fig. 4).

While there were many clones with a high similarity to known exoelectrogenic microbes, such as *G. sulfurreducens* and *Desulfito-*

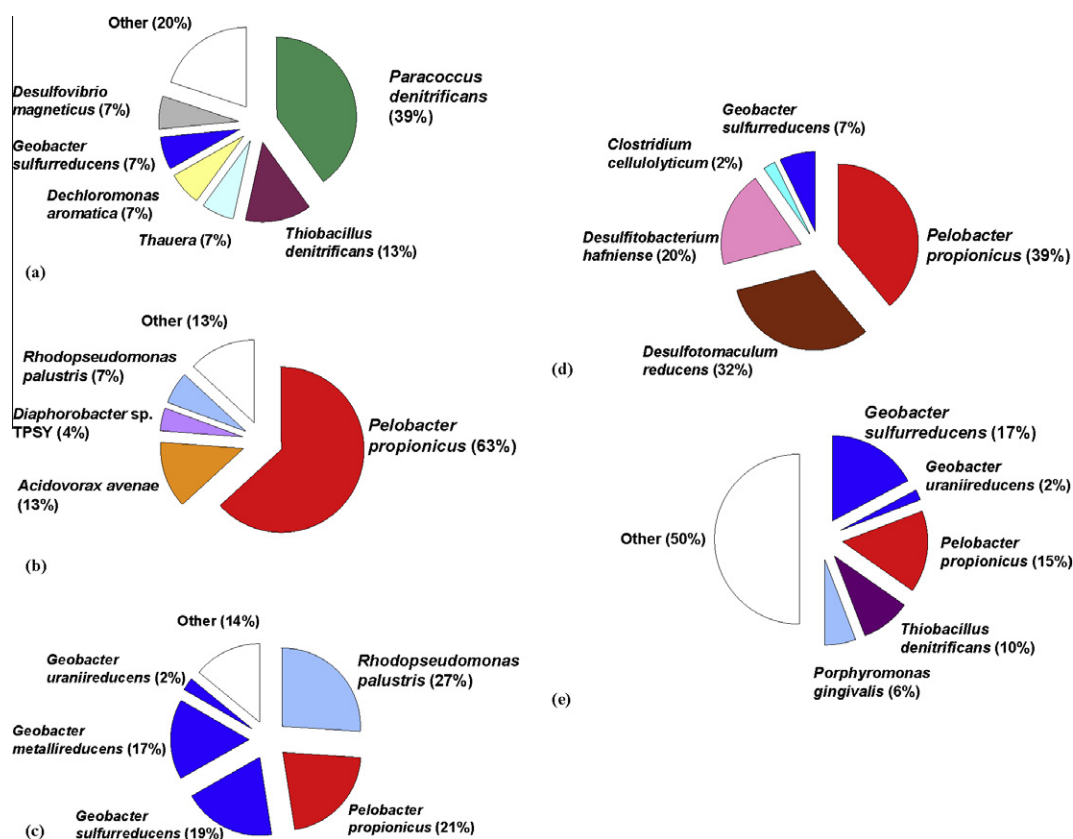
**Table 2**

Anode microbe community compositions (% representation in clone libraries), characterized by different phyla.

Substrate	Proteobacteria				Firmicutes	Others
	α	β	γ	δ		
Formic acid	41	33	5	14	0	7
Acetic acid	7	30	0	63	0	0
Ethanol	27	0	6	59	3	5
Lactic acid	0	0	0	46	54	0
Succinic acid	18	20	6	39	6	11

*bacterium hafniense*, there was an unexpected dominance of microbes such as *Pelobacter* sp. that are not known to have exoelectrogenic activity in the absence of added mediators. Other dominant strains in the clone library are known for their ability to respire using soluble electron acceptors such as nitrate. For example, formic acid-fed reactors were dominated by clones with high similarity (97% identity) to the sequenced strain *P. denitrificans* PD1222 (Fig. 4), a Gram-negative, denitrifying and anaerobic sulfide-oxidizing microorganism (Dambe et al., 2005). *P. denitrificans* was previously isolated from the anode of an MFC that converted dissolved sulfide to elemental sulfur, suggesting it has a role in both sulfide oxidation and current generation (Rabaey et al., 2006). Previous 16S rRNA community analysis of formic acid-fed reactors indentified acetogenic microbes, presumably generating acetic acid as an electron donor for *Geobacter* populations resulting in MFC electron transfer (Phuc Thi Ha et al., 2008). This process is not represented by our clone libraries.

16S rRNA gene fragments showing a high similarity (<98%) to *P. propionicus* dominated reactors fed acetic acid as an electron donor (Fig. 4). This finding is surprising as it was recently shown that another strain of this genus (*P. carbinolicus*) was incapable of transferring electrons to the anode of an MFC, although it was capable of iron reduction (Richter et al., 2007). Previously isolated *Pelobacter* spp. are also unable to oxidize acetate as an electron donor (Schink, 1984), consistent with a bioinformatic analysis showing a lack of acetate transporters in *Pelobacter* species that are found in other *Geobacteraceae* (Butler et al., 2009). *P. propionicus* has also been found to be unable to utilize formate or succinate as carbon sources (Schink, 1984). *Pelobacter* genomes also have far fewer genes associated with cytochrome expression than other members of the *Geobacteraceae* family, such as *G. sulfurreducens* (Butler et al., 2009). Certain cytochromes that have been shown to be required



**Fig. 4.** Anodic bacterial communities based on cloned 16S rRNA gene sequence distributions for MFCs fed different fermentation breakdown products. (a) formic acid, (b) acetic acid, (c) ethanol, (d) lactic acid, and (e) succinic acid. Clones showing >97% identity to previously sequenced genomes were grouped according to species.

**Table 3**

Number of clones selected for characterization of anodic communities, Good's coverage, and Shannon Diversity Index values for each community.

Substrate	No. of sequenced clones	Good's coverage	Shannon diversity index
Formic acid	46	68	2.09
Acetic acid	46	79	1.55
Ethanol	43	79	1.62
Lactic acid	41	89	1.33
Succinic acid	54	68	3.57

for microbe – anode electron transfer, such as MacA and OmcB, are absent in *P. propionicus*. Based on these physiological characteristics, it is not clear why *P. propionicus* dominated the anode of acetic acid-fed MFCs. One possibility is that *P. propionicus* has successfully evolved in the 1 year period (perhaps through horizontal gene transfer from other *Geobacteraceae*) an ability to use these substrates and produce power. Work is ongoing to isolate this microbe from the MFC and to further examine its characteristics for substrate utilization and power generation.

High percentages of clones showing significant similarity to *P. propionicus* were also found in reactors fed ethanol or lactic acid (Fig. 4). This finding is consistent with previous reports that this microbe can ferment ethanol and lactic acid, producing acetic acid (Schink, 1984; Seeliger et al., 2002). Syntrophic interactions between *Pelobacter* (fermenting initial substrates to acetate) and exoelectrogenic microbes such as *Geobacter* sp. to produce current in an MEC have been previously reported (Parameswaran et al., 2009). The microbial community in an ethanol-fed MFC previously examined by others using a combination of restriction fragment length polymorphism (RFLP) and 16S rRNA clone libraries showed very different microbe populations than those identified here (Kim et al., 2007). In that study, *Betaproteobacteria* dominated the community and only one clone showed similarity to *G. sulfurreducens*. This contrast in results could be attributed to differences in reactor architecture, operation time, and inoculum, as well as the technique used to characterize the community.

Community analysis of the succinic acid-fed reactor did not provide direct insight into the bacteria responsible for current generation. *G. sulfurreducens* is unlikely to produce current as it is unable to reduce Fe(III) using succinate (Caccavo et al., 1994). *P. propionicus* also cannot use succinate for growth (Schink, 1984). *Geothrix fermentans* has previously been shown to utilize succinate as a carbon source when Fe(III) was used as an electron acceptor (Coates et al., 1999). It is therefore surprising that we only identified one clone with significant similarity to this microbe. The CE values for the succinic acid-fed reactor are similar to those attained by acetic and lactic acid-fed MFCs. This indicates efficient conversion of succinic acid into electrical current in these reactors.

Shannon diversity index values attained for each of the reactors showed those fed succinic and formic acid were the most diverse, and the reactor fed lactic acid was the least diverse (Table 3). The higher diversity index for reactors fed formic or succinic acid could indicate a more complex pathway from electron donor to electrical current.

### 3.3. Pure culture analysis

*G. sulfurreducens* PCA and *P. propionicus* DSM 3278 inoculated into air–cathode MFCs and supplied acetic acid as the electron donor showed negligible power production. The inability to reduce the anode could be due to oxygen diffusion through the cathode, thus highlighting the important role facultative microbes play in scavenging oxygen in mixed culture MFCs. Additionally, the strains present in our reactors show 95–97% identity across the 16S rRNA gene to the type strains. Presumably the genomes of these strains

contain the genetic determinants allowing for successful exoelectrogenesis in this MFC environment. To date the successful operation of *G. sulfurreducens* PCA in both MFC and MEC has required the presence of a carbonate buffered solution (Call et al., 2009). *G. sulfurreducens* isolates with altered phenotypes have been isolated from anodes poised at low anode potentials (–0.15 V vs SHE) (Yi et al., 2009), suggesting a strong selective pressure on microbes present in this environment. The isolation of *G. sulfurreducens* and *P. propionicus* strains from these systems is continuing to characterize their exoelectrogenic capabilities in this system.

## 4. Conclusions

These results demonstrate the effects of long-term MFC operation on power production and microbial communities that develop with different substrates. The extent of the reduction in cathode performance was dependant on the electron donor. The negative impact of cathode biofilms was on maximum power densities, although overall there was a positive impact on increased CEs. The dominance of reactors fed acetic acid, ethanol, and lactic acid by members of the *Geobacteraceae* family reaffirms the mutualistic relationship between fermentation by *Pelobacter* sp. and the generation of current by *Geobacter* sp. in a MFC fed a fermentable substrate. The correlation of high power densities and relatively high *Geobacteraceae* populations in MFCs fed acetic acid, ethanol and lactic acid is particularly apparent. The anodic communities of reactors fed formic or succinic acid are much more diverse. The microbes identified to be present in these reactors offers new targets for characterizing potential novel exoelectrogenic isolates.

## Acknowledgements

The authors would like to thank Elodie Lalaurette for assisting in the maintenance of the MFCs and Deb Grove at the Penn State Genomics Core Facility – University Park, PA, for DNA sequencing. This research was supported by Award KUS-11-003-13 from the King Abdullah University of Science and Technology (KAUST) and the National Renewable Energy Laboratory (RFH-7-77623-01).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2010.05.017.

## References

- Aelterman, P., Rabaey, K., Pham, H.T., Boon, N., Verstraete, W., 2006. Continuous electricity generation at high voltages and currents using stacked microbial fuel cells. *Environ. Sci. Technol.* 40, 3388–3394.
- Balch, W.E., Fox, G.E., Magrum, L.J., Woese, C.R., Wolfe, R.S., 1979. Methanogens: reevaluation of a unique biological group. *Microbiol. Rev.* 43, 260–296.
- Bianchi, M.A.G., Bianchi, A.J.M., 1982. Statistical sampling of bacterial strains and its use in bacterial diversity measurement. *Microb. Ecol.* 8, 61–69.
- Butler, J.E., Young, N.D., Lovley, D.R., 2009. Evolution from a respiratory ancestor to fill syntrophic and fermentative niches: comparative genomics of six *Geobacteraceae* species. *BMC Genomics* 10, 103.
- Caccavo Jr., F., Lonergan, D.J., Lovley, D.R., Davis, M., Stolz, J.F., McInerney, M.J., 1994. *Geobacter sulfurreducens* sp. nov., a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. *Appl. Environ. Microbiol.* 60, 3752–3759.
- Call, D.F., Wagner, R., Logan, B.E., 2009. Hydrogen production by *Geobacter* species and a mixed consortium in a microbial electrolysis cell. *Appl. Environ. Microbiol.*
- Chae, K.J., Choi, M.J., Lee, J.W., Kim, K.Y., Kim, I.S., 2009. Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells. *Bioresour. Technol.* 100, 3518–3525.
- Cheng, S., Logan, B.E., 2007. Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells. *Electrochem. Commun.* 9, 492–496.
- Cheng, S., Liu, H., Logan, B.E., 2006. Increased performance of single-chamber microbial fuel cells using an improved cathode structure. *Electrochem. Commun.* 8, 489–494.

- Coates, J.D., Ellis, D.J., Gaw, C.V., Lovley, D.R., 1999. *Geothrix fermentans* gen. nov., sp. nov., a novel Fe(III)-reducing bacterium from a hydrocarbon-contaminated aquifer. *Int. J. Syst. Bacteriol.* 49 Pt 4, 1615–1622.
- Dambe, T., Quentmeier, A., Rother, D., Friedrich, C., Scheidig, A.J., 2005. Structure of the cytochrome complex SoxXA of *Paracoccus pantotrophus*, a heme enzyme initiating chemotrophic sulfur oxidation. *J. Struct. Biol.* 152, 229–234.
- Deng, W., Xi, D., Mao, H., Wanapat, M., 2008. The use of molecular techniques based on ribosomal RNA and DNA for rumen microbial ecosystem studies: a review. *Mol. Biol. Rep.* 35, 265–274.
- Good, I.J., 1953. The population frequencies of species and the estimation of population parameters. *Biometrika* 40, 237–264.
- Ha, P.T., Tae, Beomsoek, Chang, I.S., 2008. Performance and bacterial consortium of microbial fuel cell fed with formate. *Energy Fuels* 22, 164–168.
- Harnisch, F., Wirth, S., Schroder, U., 2009. Effects of substrate and metabolite crossover on the cathodic oxygen reduction reaction in microbial fuel cells: platinum vs. iron(II) phthalocyanine based electrodes. *Electrochem. Commun.* 11, 2253–2256.
- Hughes, J.B., Hellmann, J.J., Ricketts, T.H., Bohannon, B.J., 2001. Counting the uncountable: statistical approaches to estimating microbial diversity. *Appl. Environ. Microbiol.* 67, 4399–4406.
- Ishii, S., Shimoyama, T., Hotta, Y., Watanabe, K., 2008. Characterization of a filamentous biofilm community established in a cellulose-fed microbial fuel cell. *BMC Microbiol.* 8, 6.
- Jung, S., Regan, J.M., 2007. Comparison of anode bacterial communities and performance in microbial fuel cells with different electron donors. *Appl. Microbiol. Biotechnol.* 77, 393–402.
- Kim, J.R., Jung, S.H., Regan, J.M., Logan, B.E., 2007. Electricity generation and microbial community analysis of alcohol powered microbial fuel cells. *Bioresour. Technol.* 98, 2568–2577.
- Lalauette, E., Thammannagowda, S., Mohagheghi, A., Maness, P.-C., Logan, B.E., 2009. Hydrogen production from cellulose in a two-stage process combining fermentation and electrohydrogenesis. *Int. J. Hydrogen Energy* 34, 6201–6210.
- Logan, B.E., 2004. Peer reviewed: extracting hydrogen and electricity from renewable resources. *Environ. Sci. Technol.* 38, 160A–167A.
- Logan, B.E., Cheng, S., Watson, V., Estadt, G., 2007. Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. *Environ. Sci. Technol.* 41, 3341–3346.
- Lovley, D.R., 2008. The microbe electric: conversion of organic matter to electricity. *Curr. Opin. Biotechnol.* 19, 564–571.
- Parameswaran, P., Torres, C.I., Lee, H.-S., Krajmalnik-Brown, R., Rittmann, B., 2009. Syntrophic interactions among anode respiring bacteria (ARB) and non-ARB in a biofilm anode: electron balances. *Biotechnol. Bioeng.* 103, 513–522.
- Rabaey, K., Van de Sompel, K., Maignien, L., Boon, N., Aelterman, P., Clauwaert, P., De Schampelaire, L., Pham, H.T., Vermeulen, J., Verhaege, M., Lens, P., Verstraete, W., 2006. Microbial fuel cells for sulfide removal. *Environ. Sci. Technol.* 40, 5218–5224.
- Ren, N., Wang, A., Cao, G., Xu, J., Gao, L., 2009. Bioconversion of lignocellulosic biomass to hydrogen: Potential and challenges. *Biotechnol. Adv.*
- Richter, H., Lanthier, M., Nevin, K.P., Lovley, D.R., 2007. Lack of electricity production by *Pelobacter carbinolicus* indicates that the capacity for Fe(III) oxide reduction does not necessarily confer electron transfer ability to fuel cell anodes. *Appl. Environ. Microbiol.* 73, 5347–5353.
- Schink, B., 1984. Fermentation of 2, 3-butanediol by *Pelobacter carbinolicus* sp. nov. and *Pelobacter propionicus* sp. nov. and evidence for propionate formation from C-2 compounds. *Arch. Microbiol.* 137, 33–41.
- Seeliger, S., Janssen, P.H., Schink, B., 2002. Energetics and kinetics of lactate fermentation to acetate and propionate via methylmalonyl-CoA or acrylyl-CoA. *FEMS Microbiol. Lett.* 211, 65–70.
- Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24, 1596–1599.
- Watanabe, K., 2008. Recent developments in microbial fuel cell technologies for sustainable bioenergy. *J. Biosci. Bioeng.* 106, 528–536.
- Winker, S., Woese, C.R., 1991. A definition of the domains Archaea, Bacteria and Eucarya in terms of small subunit ribosomal RNA characteristics. *Syst. Appl. Microbiol.* 14, 305–310.
- Yang, S., Jia, B., Liu, H., 2009. Effects of the Pt loading side and cathode-biofilm on the performance of a membrane-less and single-chamber microbial fuel cell. *Bioresour. Technol.* 100, 1197–1202.
- Yi, H., Nevin, K.P., Kim, B.C., Franks, A.E., Klimes, A., Tender, L.M., Lovley, D.R., 2009. Selection of a variant of *Geobacter sulfurreducens* with enhanced capacity for current production in microbial fuel cells. *Biosens. Bioelectron.* 24, 3498–3503.
- Zhang, Z., Cheng, S., Wang, X., Huang, X., Logan, B.E., 2009. Separator characteristics for increasing performance of microbial fuel cells. *Environ. Sci. Technol.* 43, 8456–8461.