



Anode microbial communities produced by changing from microbial fuel cell to microbial electrolysis cell operation using two different wastewaters

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ABSTRACT

Conditions in microbial fuel cells (MFCs) differ from those in microbial electrolysis cells (MECs) due to the intrusion of oxygen through the cathode and the release of H₂ gas into solution. Based on 16S rRNA gene clone libraries, anode communities in reactors fed acetic acid decreased in species richness and diversity, and increased in numbers of *Geobacter sulfurreducens*, when reactors were shifted from MFCs to MECs. With a complex source of organic matter (potato wastewater), the proportion of *Geobacteraceae* remained constant when MFCs were converted into MECs, but the percentage of clones belonging to *G. sulfurreducens* decreased and the percentage of *G. metallireducens* clones increased. A dairy manure wastewater-fed MFC produced little power, and had more diverse microbial communities, but did not generate current in an MEC. These results show changes in *Geobacter* species in response to the MEC environment and that higher species diversity is not correlated with current.

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1. Introduction

Bioelectrochemical systems such as microbial fuel cells (MFCs) are devices that exploit the ability of exoelectrogenic microbes to respire through transfer of electrons outside the cell (Logan, 2009). Exoelectrogenic bacteria transfer electrons to the anode of an MFC either through direct contact (via highly conductive nanowires or membrane-associated proteins) (Lovley, 2008) or by using soluble electron shuttles (Rabaey et al., 2005). MFCs have been used to convert the energy in organic matter present in wastewaters (Ahn and Logan, 2009; Logan et al., 2008; Patil et al., 2009; Wen et al., 2009), in aquatic sediments (Miller and Oremland, 2008), and associated with plants (De Schampelaire et al., 2008) into electrical current. MFCs have also been modified to carry out a variety of tasks including hydrogen (Call and Logan, 2008), methane (Cheng et al., 2009), and hydrogen peroxide production (Rozendal et al., 2009), as well as water desalination (Cao et al., 2009). The production of hydrogen from non-fermentable substrates is made possible by electrochemical evolution of the hydrogen at the cathode in a modified MFC called a microbial electrolysis cell (MEC). As the overall reaction in an MEC is endothermic, however, additional voltage (typically in the range of 0.4–0.7 V) is applied to drive the hydrogen production reaction (Call and Logan, 2008).

In order to capture electrical energy or chemical products from these new systems using wastewater, a better understanding is needed on how the operating conditions of the system affect microbial communities (particularly exoelectrogenic populations), current densities, and recovery of the substrate as current. The complex mixture of organics present in most wastewater streams suggests that diverse microbial communities are needed to oxidize the organic matter, since many exoelectrogenic bacteria can only utilize a limited range of substrates. However, the power production can be dependent on the presence of specific strains. For example, *Shewanella oneidensis* consistently produces power densities that are much lower than mixed culture communities in MFCs (Watson and Logan, 2009) and lower current densities when compared to mixed cultures in MECs (Hu et al., 2008). MECs inoculated with *Geobacter sulfurreducens* can produce current and hydrogen as well as a mixed-culture inoculum (Call et al., 2009). The metabolic versatility of *S. oneidensis* is limited under strictly anaerobic conditions, restricting it to utilizing lactic acid as an electron donor (Biffinger et al., 2008). *G. sulfurreducens* also has a limited metabolic versatility, utilizing hydrogen and acetic acid as electron donors (Bond and Lovley, 2003), whereas *G. metallireducens* can oxidize a much wider range of organics (Lovley et al., 1993).

The performance of wastewater treatment systems can depend on creating the conditions that induce the presence of specific types of bacteria, for example a balance of filamentous and zooglyciferous bacteria in activated sludge flocs, and certain types of denitrifiers in systems operated for nutrient control (Wagner and Loy,

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2002). There is little information for bioelectrochemical systems (BES) on how the operational environment affects community structure and system performance. It is expected that MFCs used for wastewater treatment will use oxygen as the electron acceptor at the cathode, whereas MECs are operated as completely anoxic systems and evolve hydrogen at the cathode. Diffusion of oxygen into the anode chamber can affect power generation in an MFC (Zhang et al., 2009), and thus presumably the microbial community structure. Currently, there is little information on how these two different environments affect community dynamics and diversity. In addition, the anodic community is often first developed in MFCs and then switched to MEC conditions (Call and Logan, 2008). There are few reports on the effect of this shift from MFC to MEC conditions on the anodic microbial populations. In one study, *G. sulfurreducens* increased from 2% of the population in an MFC to over 70% when the anode was used in an MEC (Call et al., 2009). However, the solution conditions were not constant due to the switch from a phosphate buffer to a carbonate buffer in order to create conditions usually used to culture various *Geobacter* species. Thus, it was not known to what extent the community change was due to the buffer or the operational environment. In a different study, MFC and MEC anodic communities in a two-chamber reactor supplied acetic acid showed a reduced species diversity when changed to an MEC (Chae et al., 2008). The different conditions of the MFC and MEC are reflected by other changes in the microbial communities, as there is usually an increase in methanogenesis in MECs compared to MFCs, especially at low external voltages or high organic loading (Call et al., 2009).

To better understand the communities that develop in MFCs and MECs, the changes in the community structure were examined when anodes first used in MFCs were then used in MECs by replacing and sealing off the cathode from the air. Changes that occurred for reactors fed a single, non-fermentable substrate (acetate), as well as for reactors fed more complex organic matter sources using two different types of wastewaters (potato and dairy) were examined. The microbial communities that developed in these systems were monitored using 16S rRNA gene clone libraries and fluorescent in situ hybridization (FISH).

2. Methods

2.1. MFC/MEC reactor construction and operation

Each MFC and MEC was characterized in duplicate reactors, with results determined in triplicate. Single-chamber cubic reactors (empty bed volume 28 mL) as previously described (Call and Logan, 2008) were used for both types of reactors. A machine cut anaerobic tube (10 mL; Bellco) was glued to the top of the MEC to collect biogas. The anodes were ammonia-treated graphite fiber brushes (PANEX33 160K; Gordon Brush). Each brush (OD = 2.5 cm, L = 2.5) contained an estimated 0.22 m² of surface area with a 95% porosity (Logan et al., 2007). During MFC operation, air cathodes were used that were constructed as previously described (Cheng et al., 2006) by applying platinum (0.5 mg/cm² Pt) and four diffusion layers (PTFE) to 30 wt.% wet-proofed carbon cloth (Type B-1B, E-TEK, 3.8 cm diameter, 7 cm² of exposed surface area).

The conversion of an MFC into an MEC consisted of replacing the air cathode with an MEC cathode and sealing the reactor to ensure an anaerobic environment. MEC cathodes were constructed by applying a catalyst (0.5 mg/cm² Pt suspended in Nafion) to a circular cartridge of 30% wet-proofed carbon cloth. Reactors were sparged with N₂ for 15 min to ensure anaerobic conditions prior to each fed-batch cycle and evaluated in MFC mode before conversion to MEC mode. MECs were operated at an applied voltage of –0.9 V using a direct current power supply. MFCs were operated

at ambient temperature (23 ± 3 °C), while MECs were operated in a temperature controlled room (30 ± 1 °C).

2.2. MFC/MEC operational analysis

The voltage (E) of the reactors was measured across an external resistor (MFC: 1 k Ω , MEC: 10 Ω) every twenty minutes using a multimeter (2700; Keithley) connected to a desktop personal computer. Current (I) was calculated using $I = E/R$, where R is external resistance, with current densities normalized to the cathode projected surface area (A_{cat}). Coulombic efficiency, was calculated based on total current generation and change in substrate Chemical Oxygen Demand (COD) as previously described (Lalaurette et al., 2009). Reactors were refilled with substrates when the voltage dropped below 80 mV (~2 days for potato wastewater to ~5 days for dairy manure wastewater). To obtain polarization data in MFC mode, the external resistance was varied from 100 to 1000 Ω every 30 min over the duration of one batch cycle. Power ($P = IE$) was normalized by the cathode surface area.

Total and soluble chemical oxygen demand (TCOD and SCOD, respectively) of wastewater samples were collected before and after each batch cycle, and run in duplicate using standard methods (TNT plus COD Reagent; HACH Company). TCOd samples were run unfiltered. Samples for SCOD and high-performance liquid chromatography (HPLC) were passed through a 0.22- μm pore diameter syringe filter prior to analysis. The concentrations of volatile fatty acids and alcohols were determined by gas chromatography as previously described (Huang and Logan, 2008). Hydrogen production rate (Q_V) was calculated as previously described (Call and Logan, 2008). Volume and composition of gas produced by MECs were determined as previously described (Call and Logan, 2008).

2.3. Wastewaters

Potato processing wastewater was collected from the primary clarifier of the wastewater treatment system, and diluted with ultrapure water (Milli-Q system; Millipore Corp.) in order to lower the organic loading rate. MFCs were fed approximately 10 \times diluted potato wastewater (0.7–0.8 g/L COD, pH 6.8–7.0, Conductivity = 0.97–1.1 mS) and MECs were fed approximately 4 \times diluted wastewater (1.9–2.5 g/L COD, pH 6.4–6.8, Conductivity = 2.2–2.3 mS). Dairy manure wastewater was collected from the Berkey Creamery dairy farm at the Pennsylvania State University Campus, and was used without dilution. Wastewater served as both inoculum and substrate in all experiments. Raw wastewater characteristics are summarized in Table 1. The MFC/MECs supplied acetic acid as a substrate were filled with 50 mM phosphate buffered saline (PBS) solution and acetic acid (1 g/L) as previously described (Sellembo et al., 2010).

2.4. Bacterial community and phylogenetic analysis

DNA extraction from the anode biofilm, 16S rRNA gene amplification, cloning, and sequencing for community analysis were con-

Table 1
Composition of potato and dairy manure wastewaters.

Parameter	Wastewater	
	Potato	Dairy
pH	6.1	8.3
Conductivity (mS/cm)	5.2	2.7
TCOD (g/L)	7.7	4.3
SCOD (g/L)	2.1	0.45
Volatile Acidity (g/L of SCOD)	0.69	0.12

ducted as previously described (Kiely et al., 2009a,b). DNA was extracted using a PowerSoil DNA isolation kit (MO BIO Laboratories) according to the manufacturer's instructions. Nearly full-length 16S rRNA gene fragments of the extracted DNA were amplified by PCR using the universal bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGATC-CAGCC-3') (Winker and Woese, 1991). PCR products were purified and subsequently ligated and cloned using a TOPO TA cloning kit (Invitrogen). Plasmid extractions were carried out on these colonies using the E-Z 96 Fastfilter® Plasmid Kit® (www.omegabiotek.com/). Plasmid inserts were sequenced with the M13R primer using an ABI 3730XL DNA sequencer (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.

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 was calculated as previously described (Good, 1953). Community diversity was determined by calculating Shannon Diversity Index values for each reactor (Micheline and Bianchi, 1982) using

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

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

2.6. Fluorescent in situ hybridization (FISH)

Samples for FISH were obtained by adding several cut fibers from the anode to sterile tubes (2 mL capacity) filled with 0.5 mL of a phosphate buffered saline (PBS) solution as described previously (Kiely et al., 2009a). Cells were fixed by adding 1.5 mL of 4% paraformaldehyde and storing for 3 h at 4 °C (Amann, 1995). Following fixation, the samples were rinsed using PBS, and stored in 50% ethanol–PBS at –20 °C.

Several fibers were transferred to a sterile 200 µL PCR tube and FISH analysis was carried out as previously described (Kiely et al., 2009a). Probe GEO2 (Richter et al., 2007) which targets *Geobacter sulfurreducens*, was 5' labeled with Alexa Fluor 594 (Invitrogen) and added for a final concentration of 4.5 ng/µL. Two unlabeled helper probes HGEO2-1 and HGEO2-2 (Richter et al., 2007) were added at the same concentration as the labeled probe. Fibers were spread onto a glass slide and counterstained using 4',6'-diamidino-2-phenylindole (DAPI). Samples were examined on an Olympus BX61 epifluorescent microscope equipped with DAPI and Texas Red filter sets as described previously (Kiely et al., 2009a). Images were recorded using a DP72 digital camera and analyzed using the DP2-BSW software. Due to the substantially high background autofluorescence of the samples, direct cell counts were not possible.

3. Results and discussion

3.1. MFC performance

Analysis of dairy and potato wastewater-fed MFCs and MECs identified differences in their suitability as substrates for current

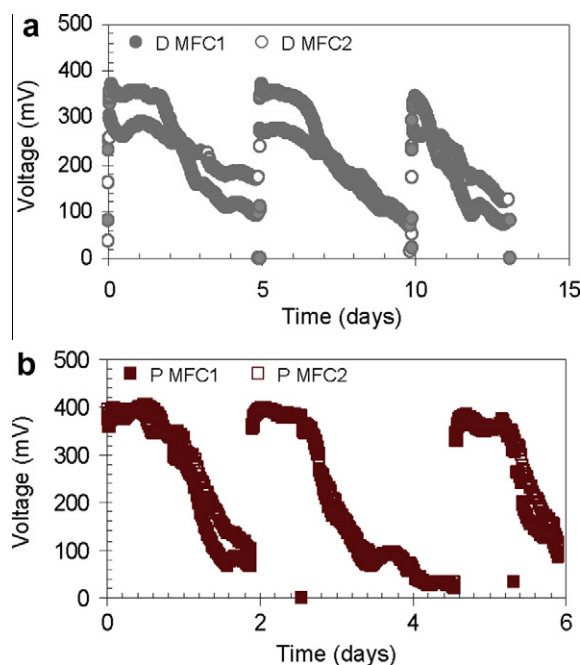


Fig. 1. Voltage generation in MFCs fed (a) dairy manure and (b) potato wastewater ($R = 1 \text{ k}\Omega$).

generation in two different bioelectrochemical systems. MFCs operated in fed-batch mode for 90 days produced maximum voltages of ~400 mV (1 k Ω external resistor) (Fig. 1a and b). Fed-batch cycle times varied from ~2 days for potato wastewater to ~5 days for dairy manure wastewater. TCOD removals were 89% for MFCs fed potato wastewater, and 70% for the dairy manure wastewater (Table 2). Coulombic efficiencies (1 k Ω resistor) were higher for the MFC fed potato wastewater (21.1%) than the dairy manure wastewater (11.7%) (Table 2). Both wastewaters generated power in MFCs, with 217 mW/m² for the potato wastewater and 189 mW/m² for the animal wastewater (Fig. 2). These power densities are higher than those produced from MFCs fed other unbuffered wastewaters (conductivities ~1 mS/cm) (Feng et al., 2008; Huang and Logan, 2008) in identical MFCs. However, they are much lower than 2400 mW/m² produced in these same MFCs fed well-buffered solutions of acetate with high solution conductivity in the region of 7 mS/cm (Logan et al., 2007).

3.2. MEC performance

Following MFC tests, cathodes were changed and the anodes were used in reactors operated as MECs. MECs fed potato wastewater exhibited reproducible results in terms of current generation, biogas production, Coulombic efficiency, and TCOD removal. At an applied voltage of 0.9 V, MECs fed potato wastewater produced

Table 2
Performance comparison of potato and dairy manure wastewater-fed MFCs and MECs.

Mode	Parameter	Wastewater	
		Potato	Dairy
MFC	TCOD removal (%)	89	70
	CE (%)	21	12
MEC	TCOD removal (%)	79	–
	CE (%)	80	–
	% H ₂	73	–
	Q (m ³ -H ₂ /m ³ -d)	0.74	–

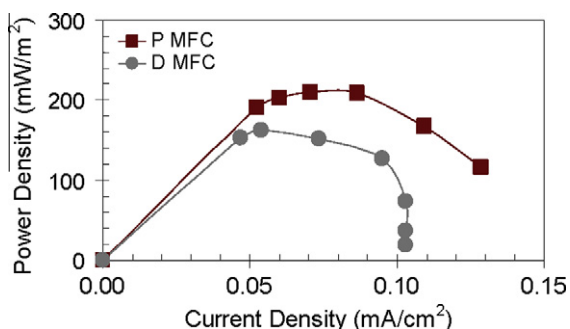


Fig. 2. Power density curves of dairy manure (D MFC) and potato (P MFC) wastewater-fed MFCs normalized to cathode projected area.

~4.5 mA (0.64 mA/cm²) of electrical current and 0.74 m³-H₂/m³-d (Fig. 3, Table 2). The biogas produced by potato wastewater MECs was on average 73% H₂, 13% CH₄, and 14% CO₂. The Coulombic efficiency was 80%, with a total COD removal of 79% (Table 2).

MECs fed dairy manure wastewater did not produce measurable quantities of biogas. Current production was very low (<1 mA) at an applied voltage of 0.9 V, suggesting that the microbial community present on the anode was unable to donate electrons at a significant rate under MEC conditions (Fig. 3a, Table 2).

3.3. Potato wastewater as a substrate for MFCs and MECs

Potato wastewater was a suitable substrate for both MFCs and MECs. This could be due in part to the relatively high concentrations of volatile fatty acids (VFAs), and a relatively high solution conductivity of the potato wastewater (Table 1). Previous MFC tests using a paper plant wastewater showed bimodal power densities that were attributed to a progression from soluble COD utilization to particulate COD degradation (Huang and Logan, 2008). A similar situation may have occurred here for the potato wastewa-

ter as there was an initial peak in current observed in the first day of batch operation, followed by a secondary peak before a rapid reduction in current generation. This first peak could have been due to utilization of easily assimilated compounds such as VFAs present in the potato wastewater, with the secondary peak a result of the utilization of more complex organics. In addition, current generation may have been stimulated by hydrogen recycling from the cathode to the anode. The potato wastewater fed MECs had an electron recovery similar to that achieved by acetate fed reactors (Call and Logan, 2008). These encouraging results with the potato wastewater demonstrate feasibility of efficiently treating this unbuffered, raw wastewater with MECs.

Anode communities in the MFCs fed potato wastewater were dominated by various *Geobacter* species. Microbial communities on the anode of the MFCs and MECs fed potato wastewater were dominated by *Geobacteraceae* (>60% of all clones) (Fig. 4). *G. lovleyi* populations were present in both MFC and MECs, representing 14% and 21% of the clones sequenced. Strains with significant similarity to *G. sulfurreducens* were only identified in the MFC and represented 37% of the total bacterial community. These *Geobacter* isolates have previously been shown to generate high current densities in MFCs (Bond and Lovley, 2003) and high current densities and hydrogen recoveries in MECs (Call et al., 2009).

3.4. Dairy manure wastewater as a substrate for MFCs and MECs

Although dairy manure wastewater was suitable for treatment in MFCs, very little current or hydrogen was produced using this wastewater in MECs (Fig. 3). In contrast to potato wastewater fed MECs, dairy manure wastewater fed MECs had relatively few *Geobacteraceae* (<12%). We therefore infer that the low current production of the MEC fed dairy manure wastewater was in part attributable to the near absence of *Geobacter* in the microbial community. Community profiles for MECs that generate appreciable current densities have shown a significant proportion of *Geobacter* in the anodic community (Call and Logan, 2008; Chae et al., 2008). For example, in a recent study where MEC anodes were set at different potentials, the microbial communities at a set potential of -0.15 vs SHE were dominated by *G. sulfurreducens* (>97% similarity) (Torres et al., 2009). Anode communities that developed at higher set potentials in their study were more diverse, but were unable to produce significant current densities. This suggests that the presence of various *Geobacter* species was needed for successful MEC operation. However, these previous MEC studies were conducted with a single substrate (acetate), not complex sources of organic matter. In addition, the presence or absence of *Geobacter* does not explain the failure of the anode from the MFC to produce current in the MEC, when power was successfully generated by this same anode in the MFC. We hypothesize that oxygen utilization may have been critical to the functioning of the anode community with the dairy manure wastewater, and thus that the other microorganisms in the community were an essential link to substrate degradation and current generation under MEC operating conditions.

The wastewater fed MECs also showed the presence of clones with similarity to fermentative bacteria including *Pelobacter propionicus* and *Geothrix fermentans*. The presence of these two bacteria was likely a result of the need for hydrolysis and fermentation of the complex organic matter prior to current generation. In order for *G. sulfurreducens* to produce power, appreciable acetate or hydrogen would have to have been produced (Caccavo et al., 1994). *G. fermentans* may also have been responsible for current generation in potato wastewater. This microorganism has been shown to be metabolically versatile and it can generate electricity using electron shuttles in MECs with an anode set at +200 mV (vs Ag/AgCl) (Bond and Lovley, 2005).

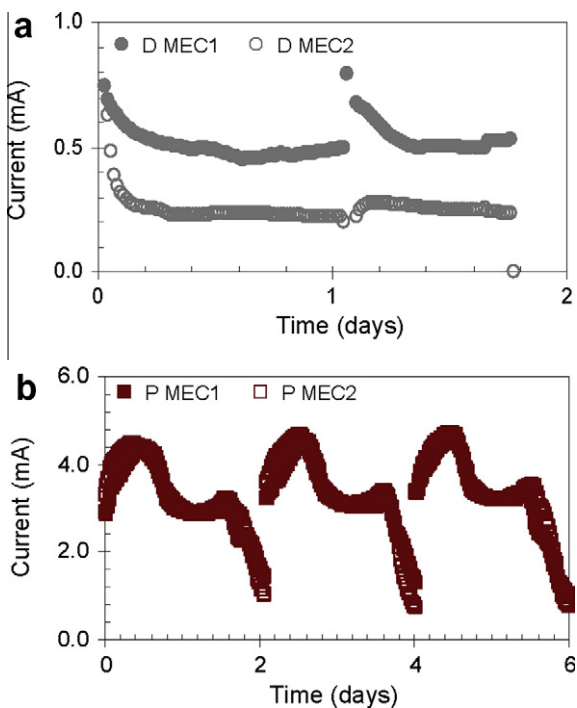


Fig. 3. Current produced in MECs fed (a) dairy manure and (b) potato wastewater.

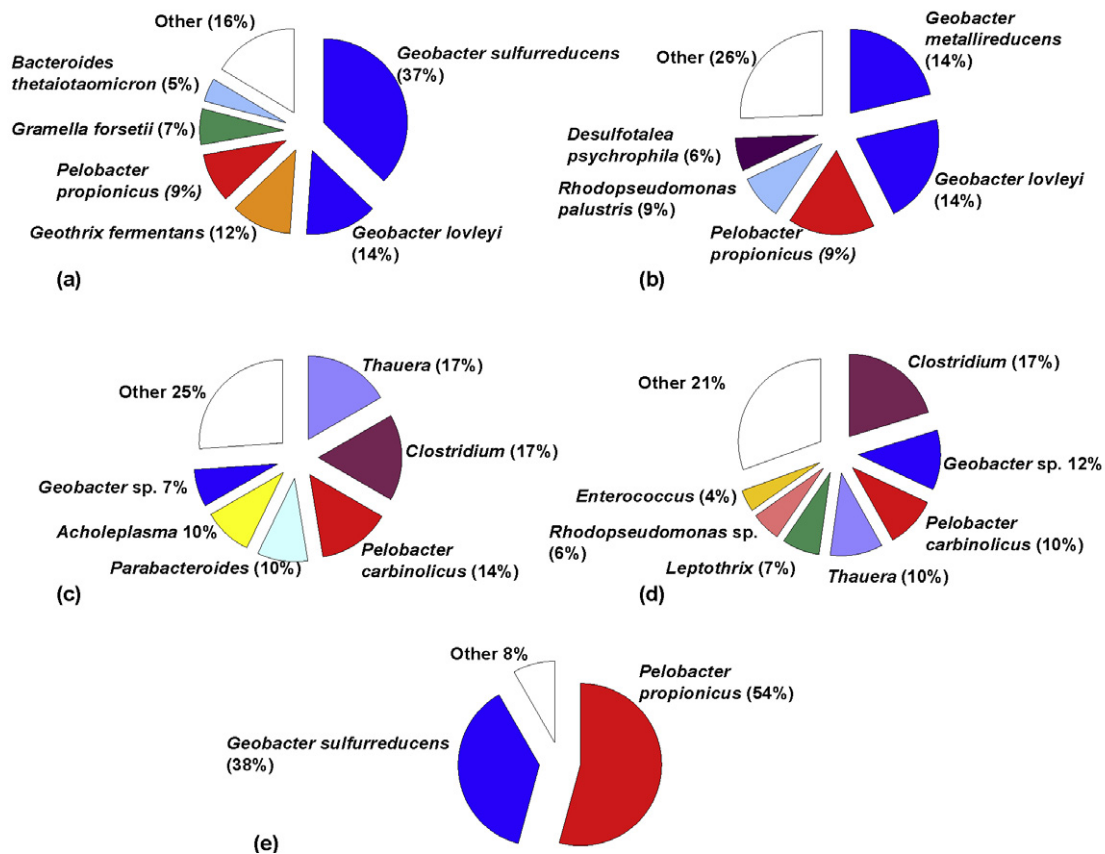


Fig. 4. Anodic bacterial communities based on cloned 16S rRNA gene sequence distribution for MFCs and MECs fed different substrates. (a) MFC + potato WW, (b) MEC + potato WW, (c) MFC + dairy WW, (d) MEC + dairy WW, (e) MEC + acetic acid.

3.4.1. 16S rRNA gene characterization of the acetic acid fed MEC anode bacterial community

Characterization of the anode biofilm from the MEC fed acetate showed it was dominated by *Geobacteraceae*. *P. propionicus* clones made up 54% of the community, and *G. sulfurreducens* accounted for 38% of the population. There were also two other species represented by single clones, showing significant similarity to *Alkaliphilus oremlandii* (>88%) and *Dechloromonas aromatica* (>95%) (Fig. 4e). These results are similar to those previously obtained for the anode community of an MFC with a similar reactor archi-

ture that was also fed acetic acid, where the community was also dominated by clones with similarities to *P. propionicus* and facultative *Betaproteobacteria* (Kiely et al., 2009b).

3.5. FISH analysis for *G. sulfurreducens* on MEC anodes supplied potato or dairy manure wastewater

To corroborate the 16S rRNA gene clone analysis indicating that *Geobacter* species were abundant in the potato wastewater-fed communities, and not the dairy manure wastewater reactors, an-

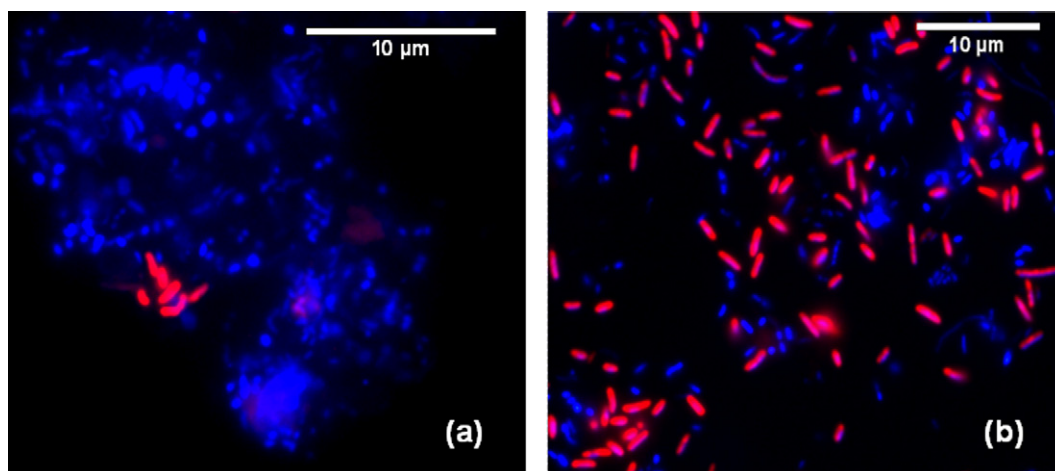


Fig. 5. FISH images of MEC anode fibers spread onto a slide from (a) dairy manure or (b) potato wastewater. Blue indicates all bacteria and archaea (DAPI) and red shows *G. sulfurreducens*. Scale bar represents 10 µm.

Table 3

Number of clones selected for characterization of anodic communities, Goods coverage, and Shannon diversity index for each community.

Reactor type	No. of sequenced clones	Goods coverage (%)	Shannon diversity index
MFC + dairy WW	50	56	2.21
MEC + dairy WW	81	57	2.47
MFC + potato WW	44	68	2.03
MEC + potato WW	47	64	2.27
MEC + acetic acid	47	92	0.86

odes of the dairy and potato wastewater supplied MECs were examined using FISH. The anode biofilm present on the potato wastewater MEC was found to be highly infused with *G. sulfurreducens* cells, whereas the dairy manure wastewater fed MEC showed a lower abundance of *G. sulfurreducens*. The *G. sulfurreducens* cells were shown to be homogeneously dispersed throughout the potato wastewater anode, whereas those identified in the dairy manure wastewater anode formed distinct clumps or aggregates of cells (Fig. 5a and b).

3.6. Effect of MFC–MEC shift on microbial diversity

The Shannon diversity index values for the MFC anode communities are comparable to previous values calculated for the conversion of the fermentation breakdown products, formic acid (2.09) and succinic acid (2.22), into electrical current (Kiely et al., 2009b). These diversity values are much higher than those attained for reactors fed substrates which show a direct pathway to acetic acid metabolism (acetic acid, ethanol, lactic acid) and reflect the processes required to convert these complex organics into electrical current. It is interesting to note that MECs fed mixed organic substrates (dairy, potato) have greater community diversity when compared to their MFC equivalents (Table 3). This increased diversity could indicate a potential role for O₂ respiring microbes in the oxidation of certain complex organics into breakdown products suitable for fermentative and exoelectrogenic populations. This is in contrast to the higher diversity previously characterized for acetic acid fed MFCs (Chae et al., 2008) when compared to their equivalent MEC anode communities.

4. Conclusions

This is the first time that the communities have been examined for electrodes used in both MFCs and MECs for wastewater sources. MECs supplied with dairy manure wastewater produced negligible current and gas, which could be directly attributed to the relatively low numbers of clones with similarity to *Geobacter* species identified on the anode of this system. This work reaffirms the importance anodic *Geobacteraceae* populations play in the generation of current in MFCs and particularly in gas production in MECs.

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