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BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

Microbial community differences between propionate-fed microbial fuel cell systems under open and closed circuit conditions

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Abstract We report the electrochemical characterization and microbial community analysis of closed circuit microbial fuel cells (CC-MFCs) and open circuit (OC) cells continuously fed with propionate as substrate. Differences in power output between MFCs correlated with their polarization behavior, which is related to the maturation of the anodophilic communities. The microbial communities residing in the biofilm growing on the electrode, biofouled cation-exchange membrane and anodic chamber liquor of OC-and CC-MFCs were characterized by restriction fragment length polymorphism screening of 16S rRNA gene clone libraries. The results show that the CC-MFC anode was enriched in several microorganisms related to known electrochemically active and dissimilatory Fe(III) reducing bacteria, mostly from the *Geobacter* spp., to the detriment of *Bacteroidetes* abundant in the OC-MFC anode. The results also evidenced the lack of a specific pelagic community in the liquor sample. The biofilm growing on the cation-exchange membrane of the CC-MFC was found to be composed of a low-diversity community dominated by two microaerophilic species of the *Achromobacter* and *Azovibrio* genus.

Keywords Microbial fuel cell · Propionate · Microbial community · *Geobacter*

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Introduction

Some bacteria, termed electrochemically active bacteria (EAB, Chang et al. 2006), can use an electrode (poised at a convenient potential) as a terminal electron acceptor, either by direct physical contact (Kostka et al. 2002) or through the use of soluble electron shuttling compounds (Roller et al. 1984). This fact made it possible to develop microbial fuel cells (MFCs), bio-electrochemical devices capable of converting the chemical energy stored in organic matter to electrical energy through reactions achieved by microorganisms. In such system, EAB are able to degrade organic matter producing in return electricity, water, and CO_2.

Due to current energy and water issues, there is an increasing awareness of the need to improve wastewater treatment processes both in efficiency and cost. Wastewaters usually contain organic matter that can be treated by MFCs with concomitant electricity generation (Kim et al. 2004; Oh and Logan 2005), and previous estimations show that MFC technology could reduce energy costs in conventional treatments processes by 50% and potentially yield 50–90% less solid waste to be disposed of (Holzman 2005; Kim et al. 2007). Although organic waste removals of up to 80% (Liu et al. 2004; Min et al. 2005) and

coulombic efficiencies as high as 80% (Kim et al. 2005) have been reported, MFC technology is far from mature, especially in comparison with anaerobic treatment processes (Rabaey and Verstraete 2005).

Propionate is a major intermediate in the anaerobic degradation of organic matter (Schink 1997), being the precursor of up to 35% of the methane produced in anaerobic wastewater treatment system (Koch et al. 1983). Thus, propionate may also play an important role in the metabolic network of MFC systems treating wastewaters. Although it has been shown that electrical current could be derived from propionate degradation employing MFCs using pure (Holmes et al. 2004) or mixed (Jang et al. 2010; Oh and Logan 2005) cultures, there is currently a lack of experimental data regarding the nature of propionate-degrading communities in MFCs systems, and their electrochemical performance.

If MFC technology is to be used in future wastewater treatment processes, a suitable understanding of the ecology of the microbial communities dwelling in the different environments of the system, and an improved knowledge of the role of propionate in the system should be attained to better control the biological processes in charge of waste removal with concomitant electricity production and improve MFC design.

In this paper, we report the operation, electrochemical characterization, and microbial community analysis of the different environments of MFCs continuously fed with propionate as substrate. We included open circuit (OC)-MFCs in the study to be able to discern the populations specifically related to current generation from those selected by the reactor configuration and operation, and designed the system taking into account that practical applications of MFC technology for wastewater treatment will most likely require continuous flow operation. Furthermore, the use of a cation exchange membrane (CEM) to separate the anodic (anaerobic) and cathodic (aerobic) environments was preferred over the membraneless-MFC configuration, often employed in studies of MFC technology treating wastewaters (Aldrovandi et al. 2009; Ghangrekar and Shinde 2008), for ease of microbial community analysis.

Materials and methods

MFC construction and operation

Six dual-chamber MFCs were constructed as previously described (Jang et al. 2010) with transparent polyacrylic plastic consisting of an anode and a cathode compartment of equal volume (5×1×1 cm). Each compartment contained two pieces (4.5×1×0.5 cm) of graphite felt (GF series, Electrosynthesis, Amherst, NY, USA) as electrodes, but the graphite felt used for the cathode electrode was coated with a Nafion-platinum solution (0.3 mg/cm^2 final platinum coating). The anode and cathode compartments were separated by a Nafion 450 cation-exchange membrane (DuPont, Wilmington, DE, USA), and platinum wires (0.5 mm diameter) of 3 cm length were utilized as leads for both electrodes. MFCs were operated by continuously feeding synthetic propionate–wastewater medium to the anode compartment using peristaltic pumps (Watson-Marlow, Campel, UK) at 0.43 ml/min. The cathode compartment was continuously fed with air-saturated 50 mM phosphate buffer (pH 7.0). The MFCs were installed in a chamber that was temperature-controlled to 30°C, and the medium reservoir was connected to a nitrogen-containing gas-tight bag (SKC Inc., Eighty Four, PA, USA). The synthetic propionate–wastewater medium contained 5 mM sodium propionate, 0.226 g/l NH_4Cl, 0.077 g/l $MgCl_2$, 0.015 g/l $CaCl_2$, 0.001 g/l $FeCl_3{\cdot}6H_2O$ and 0.0234 g/l $MnCl{\cdot}4H_2O$, and trace minerals (Lee et al. 2003). After autoclaving at 121°C for 15 min, cooling and gassing with oxygen-free nitrogen for at least 2 h, phosphate buffer (5 mM, pH 7.0) and $NaHCO_3$ (0.42 g/l) were added through a sterilized filter. MFCs were inoculated with previously homogenized anaerobic sludge obtained from a nearby brewing company (Gwangju, Korea). Fresh inoculum was prepared as follows: the sludge was diluted 1:100 with propionate medium and was re-circulated through the MFCs during a 20 h period, after which the flow was stopped for a period of 63 h to foster anodophilic biofilm formation. Next, the medium flow (sterile propionate medium) was started again and maintained for 2 weeks to establish a stable anaerobic propionate-degrading microbial community. Later, three of the MFCs were set to close circuit mode (CC) at 10 kΩ resistance for 4 days, after which the resistance was changed to the final 30 Ω to maximize current output and propionate removal. The other three MFCs were kept at OC mode as controls.

Measurements and calculations

The drops in potential across the external resistor were measured using a digital multimeter (Keithley Instruments, Cleveland, OH, USA) and recorded on a personal computer through a data acquisition system (ExceLINX, Keithley Instruments). The measured potential was converted to current according to Ohm's law ($V=I\times R$). Current was also converted to coulombs (A=C/s), and coulombic efficiency was calculated by dividing observed coulombs by theoretical coulombs, which were determined from the amount of substrate consumed by the MFCs. Current and power densities were calculated based on the actual volume

(5 cm^3) of anode compartment and apparent surface area (20 cm^2) of anode electrode. Propionate consumption was analyzed three times over a 15-day period by measuring its concentration in inflow and outflow triplicate samples by high-performance liquid chromatography (HPLC) (Younglin Co., Korea) using an Aminex HPX-87 H column (Bio-Rad Laboratories, Hercules, CA, USA) equipped with a UV detector (at 210 nm), and expressed as a percent (fraction of propionate consumed). The CC-MFCs polarization behavior was analyzed by applying different resistances until a current plateau was reached.

Sampling of MFCs for community analysis

After a period of enrichment and characterization, the OC- and CC-MFC (operated at 30 Ω) showing the highest voltages were destructively sampled. The anodes were cut with a sterile scalpel into pieces (approximately 5×5 mm), and the lower and upper sections corresponding to the regions closer to the inflow and outflow ports were discarded. Each final sample was composed of two pieces with not-neighboring original locations. The same procedure was taken while sampling the CC MFC Nafion membrane. The membrane and electrode samples were washed in 1 ml of phosphate buffered saline and twice shaken for 5 min at 250 rpm. In the case of the electrode samples, the supernatant from both washing–shaking steps was collected, centrifuged, and stored as "liquor" samples. Samples were stored in freezer until further use.

DNA extraction and 16S rRNA gene library construction

Total DNA was extracted from three different aliquots of inoculum, membrane, liquor, and anode samples, using the *PowerSoil* DNA Isolation Kit (Mo Bio Laboratories Inc. Carlsbad, CA, USA) according to the manufacturer's instructions. The products were analyzed by agarose gel electrophoresis, and equal amounts of DNA from each aliquot were pooled. The resulting DNA samples were then used to amplify the 16S rRNA genes from *Bacteria* present using the Bulk AccuPower PCR Premix, (Bioneer, Deajon, Korea) and primer pair 27f-1492R (Weisburg et al. 1991). The products of triplicate PCR amplifications from each pooled sample were joined and purified using the GeneClean Turbo DNA purification Kit (Quiogene Carlsbad, CA, USA), before cloning into pGEM-T Easy Vector (Promega, Madison, WI, USA). The resulting plasmids were used to transform competent *Escherichia coli* DH5α cells (TaKaRa, Japan), thus obtaining clone libraries from the bacterial 16S rRNA genes present in the inoculum, CC-MFC membrane, and both anode and liquor originating from the OC and CC MFCs sampled.

Restriction fragment length polymorphism and phylogenetic analyses

Random clones from each library were chosen for further analysis; bacteria containing the plasmid were lysed in 5 μl of deionized water for 20 min at 94°C. The lysate was used for subsequent amplification of the insert using primers M13rv and M13fw. Inserts of the correct size were cut overnight with 0.25 μl each of restriction enzymes *Xho*I and *Hae*III (New England BioLabs, Ipswich, MA, USA) separately in a final volume of 40 μl. The products were then separated through a 3% MetaPhor agarose gel (TaKaRa). Clones with identical restriction patterns were grouped, and one clone from each group with more than one member was regrown in Luria–Bertani media; its plasmid was extracted using the Exprep Plasmid SV purification Kit (GeneAll Biotechnology, Seoul, Korea) and sequenced (SolGent, Daejeon, Korea). The sequences obtained were edited using BIOEDIT (Hall 1999). The online programs CHECK_CHIMERA (Cole et al. 2005) and Bellerophon (Huber et al. 2004) were used to rule out the presence of chimeric sequences. Phylogenetic classification of the sequences was obtained using the RDPII Bayesian classifier using a 70% threshold (Cole et al. 2005). The phylogenetic relationships between the *Geobacteraceae* sequences detected in the study and a representative set of sequences obtained from the RDP II database were assessed by constructing a bootstrapped phylogenetic tree using the neighbor-joining method based on Kimura's two-parameter distances (Kimura 1980). The phylogenetic and rarefaction analysis as well as the diversity indices were obtained using the R package *ape* (Paradis et al. 2004).

Nucleotide sequence accession numbers

The sequence data has been deposited to the GenBank database under accession numbers GU591498–GU591545.

Results

Performance of the MFCs

The voltage recorded for all six cells (under OC mode) stabilized in the range of 0.7–0.8 V within 2 weeks. Then, three cells were changed to CC mode. In accordance with the MFC rationale, when fuel feeding to the cells was stopped, due to routine changes of medium reservoirs or sampling, the potential developed across the electrodes dropped in both OC cells and CC-MFCs, but immediately recovered as the feeding was resumed. The characterization of the MFCs was started after a stable current production was attained. The maximum

voltage recorded for a single CC-MFC (MFC 2) was 47.4 mV at the operating resistance of 30 Ω, which can be translated to 1.58 mA (316.0 A and 15.0 W/m^3 of anodic chamber volume, or 790 mA and 37.4 mW/m^2 of apparent surface area of anode electrode). In order to gain a better understanding as to why one of the CC-MFCs (MFC 2) consistently produced more electricity than its siblings, we assessed their polarization behavior by increasing the resistance in a stepwise fashion. The results (Fig. 1) showed that the observed differences in performance correlated with their general polarization behavior. It was also observed that the power obtained by the CC-MFCs could be increased by employing higher resistances. CC-MFC-2 was then set to 500 Ω resistance during 5 days, resulting in a steady performance close to 340 mV (46.24 W/m^3 of anodic chamber volume or 115.6 mW/m^2 of apparent surface area of electrode). The consumption of propionate was measured three times during 15 days; the influent and effluent of the OC cells and CC-MFCs showing the highest voltage (MFC-6 as OC mode delegate and MFC-2 as CC mode delegate, respectively) were analyzed by HPLC. No detectable concentrations of acetate (<0.1 mM) were observed in the effluent. The average propionate consumption rate for MFC-6 (OC mode) was 2.5±1.3%, while that of the MFC-2 (CC mode) was 6.0±1.1%, which corresponded to a coulombic yield of 31.5±0.6%.

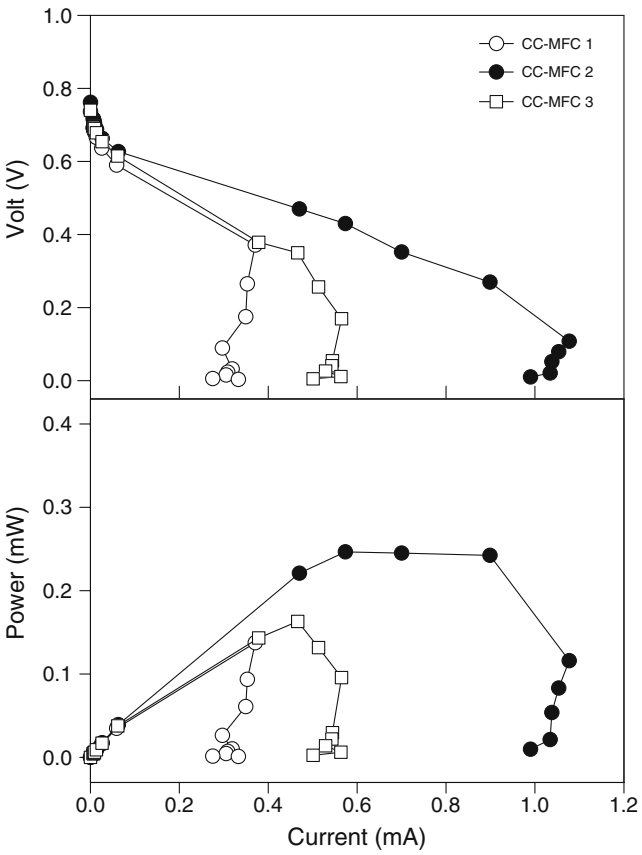

Fig. 1 Polarization (*up*) and IV (*down*) curves for CC-MFCs obtained by varying the external resistance, and plotted vs electrical current obtained

Microbial communities in the different environments of the MFCs

After current monitoring and coulombic yield analysis were determined, samples from both the anode biofilm and liquor of each of OC cell and CC-MFC (again MFC-6 and 2) were destructively taken. The CEM of the CC-MFC was sampled as well. With the intention of studying the major constituents of the microbial communities present in the different environments, bacterial 16S rRNA gene libraries were constructed from DNA derived from the samples and the initial inoculum, and analyzed by restriction fragment length polymorphism (RFLP). Clones showing equal RFLP patterns were grouped as operational taxonomic units (OTUs), and one sequence from each group that was comprised of at least two clones was sequenced. In this manner, 52–61 clones from each bacterial library (64 clones from the inoculum) were analyzed. The rarefaction curves (not shown) constructed to estimate the sampling effort as expected did not reach saturation, indicating that the number of clones analyzed was not sufficient to cover all the OTUs that could be retrieved from the samples. However, the dominant members of the communities were sampled, and the abundances recorded reflect their dominance. The non-exhaustive nature of the survey and the inevitable cross-contamination of the communities living in the different environments sampled (i.e., liquor-dwelling bacteria on the anode samples, membrane biofilm bacteria detaching and being retrieved in the liquor samples, etc.) require a conservative analysis of the results.

The most dominant member of the inoculum (EBL2, *Syntrophaceae*, 32.7%), likely an H_2-producing fatty acid-oxidizer, similar to those commonly present in methanogenic environments (Diaz et al. 2006), was not recovered from the different environments studied, probably affected by the dramatic change in retention time of the environment (Shigematsu et al. 2006). However, the microbial communities that evolved from the initial inoculum were still dominated by sequences most similar to uncultured organisms commonly found in wastewater treatment or anaerobic environments (Table 1).

One *Geobacteraceae* sequence (EBL40) was observed in high abundance in the anode of the OC cell and CC-MFC (15.5% and 19.7%, respectively). Nevertheless, the CC-MFC anode presented three other *Geobacteraceae* OTUs (EBL30, EBL42, and EBL29), and the total percentage of OTUs from such group rose to 47.5%. Other abundant sequences such as those from *Acidaminococcaceae* (EBL36, 8.2%) and *Clostridiaceae* (EBL22 and EBL25, 6.56%) were only found in

Table 1 The diversity comparison of gene libraries derived from different environments sampled in OC cell and CC-MFC

Classification	Samples (%)				
	OC anode	CC anode	OC liquor	CC liquor	CEM
Rikenellaceae	–	3.28	6.78	6.90	7.69
Alcaligenaceae	–	–	5.08	1.72	19.23
Nitrospiraceae	1.72	1.64	1.69	3.45	–
Rhodobacteraceae	–	1.64	1.69	–	–
Spirochaetaceae	1.72	1.64	–	–	–
Bacteroidetes	22.41	9.84	10.17	1.72	23.08
Acholeplasmataceae	–	–	–	3.45	–
Geobacteraceae	15.52	47.54	5.08	3.45	–
Rhodocyclaceae	31.03	4.92	44.07	74.14	32.7
Acidaminococcaceae	–	8.20	–	–	–
Clostridiaceae	–	6.56	–	–	–
Ideonella	–	–	6.78	–	–
Others	27.6	13.11	18.64	5.17	17.31

OC open circuit, *CC* closed circuit, *CEM* cation exchange membrane, (–) not detected

the anode of the CC-MFC. Furthermore, one *Rikenellaceae* sequence (EBL27), highly similar to a dissimilatory iron-reducing enrichment clone (Lin et al. 2007), was only retrieved from the CC-MFC anode library (3.28%). On the other hand, *Bacteroidetes* sequences were enriched in the OC cell anode surface (22.4%) in comparison to that of the CC-MFC (9.8%).

The enrichment of *Geobacteraceae* has been widely observed in the anodophilic communities of MFCs (Bond et al. 2002; Gregory et al. 2004; Jung and Regan 2007) and is thought to be due to the ability of these organisms to reduce Fe(III) oxides, which hold significant similarities with graphite electrodes (Gregory et al. 2004). When the phylogeny of the *Geobacteraceae* sequences retrieved was analyzed (Fig. 2), it was observed that all of them lay within the *Geobacter* genus. While EBL42 is related to *Geobacter hephaestius*, the rest of the sequences were placed inside the cluster containing the well-known *Geobacter sulfurreducens* and *Geobacter metallireducens*. EBL29 is most similar to *G. sulfurreducens,* but EBL30 and EBL40 were clustered separately from the other species of the group, indicating that they may represent novel species.

The library derived from the biofilm fouling the CEM was dominated by only three OTUs, related respectively to *Achromobacter* (EBL47, 19.2%), *Azovibrio* (EBL16, 32.7%), and *Bacteroidetes* (EBL15, 23.08%). The liquor libraries were dominated by *Zoogloea* sequences (74.1% and 44.1% for the OC cell and CC-MFC, respectively).

Discussion

The maximum current output attained during this study using continuous flow MFCs fed with propionate–waste-

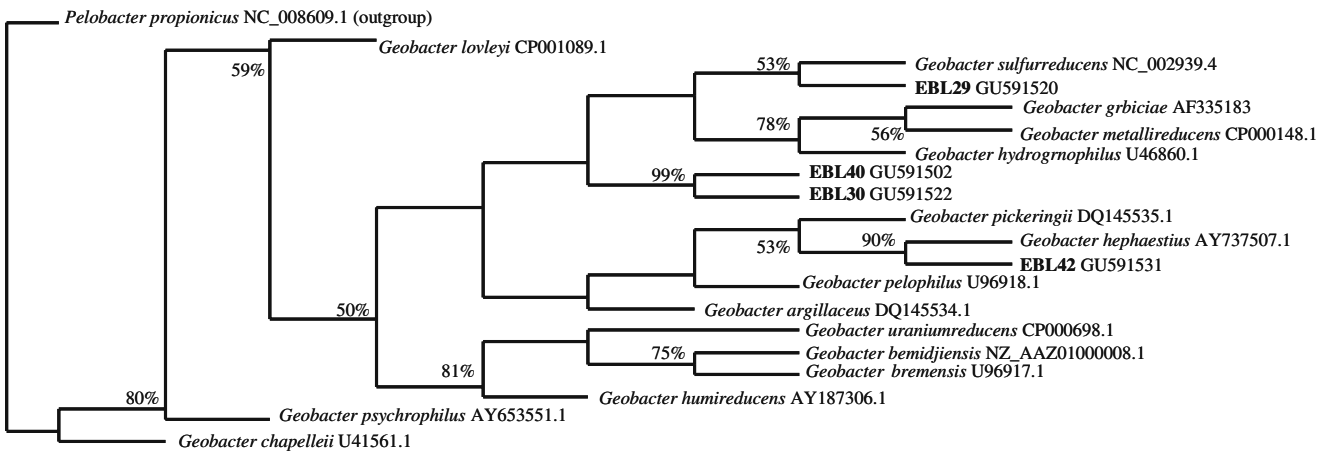

Fig. 2 Phylogenetic tree depicting the relationship between *Geobacteraceae* sequences obtained in this study and representative sequences from the *Geobacter* genus. Supporting bootstrapping values higher or equal to 50% are shown

water was 36.0 A/m^3 of anode volume, equivalent to 15.0 W/m^3 at 30 Ω. However, it was shown that at least a threefold increase in power output could be obtained by optimizing the external resistance applied at 500 Ω. Although other studies have shown power outputs up to 216 W/m^3 in continuous flow MFCs (Rabaey et al. 2003), such systems used a ferricyanide solution at the cathode. The highest power outputs reached by more sustainable systems were around 50 W/m^3 of total cell volume (Cheng et al. 2006), similar to the 55 W/m^3 reached in this study. The polarization curve of a MFC may offer valuable insights regarding the maturation and activity of its resident microbial community, especially of the anodophilic community (Aelterman et al. 2006). The shape of polarization curve (i.e., *I–V* curve) is related to the presence of the different potential losses in the system (O'Hayre et al. 2006); the initial steep voltage drop is associated with the activation losses, followed by a linear loss related to the ohmic losses (i.e., resistance of the electrolyte), and eventually a sharp drop indicates the reaching of mass transfer limitations (i.e., limitations on the influx of substrate and/or removal of wastes). Aelterman et al. (2006) showed that microbial communities growing on MFCs could overcome both diffusion and activity limitations over time. They noticed the same mass transfer issues such as early steep mass transfer losses and voltage return at very low resistances that were observed in this study (Fig. 1) and showed that an increase in flow rate did not mend such behavior. The authors concluded that such losses must occur mainly within the anodic biofilms and that the observed long-term disappearance of those adverse effects implied that the anodic biofilm eventually improved the mass transfer limitations, probably through the evolution of an optimized architecture and (or) composition. In our study, the construction, operation, and initial general behavior (not shown) of all three CC-MFCs was equal, and the OC cells maintained similar high voltages (higher than 0.7 V) during the experiment. Therefore, the differences observed in the polarization curves are most likely due to differences in the bacterial communities present in the system and not to operational or design flaws (i.e., oxygen intrusion). The lower slope observed in the ohmic region of MFC-2 is directly related to a higher activity on the anode (O'Hayre et al. 2006), probably arisen from a more complete colonization of the electrode by EAB. Moreover, the different starting points of the mass transfer losses observed between the three MFCs are, following rationale expressed by Aelterman et al. (2006), related to the different maturation of the anodic biofilms, and how they cope with the mass transfer issues. The information derived from the polarization curves prompted us to choose MFC-2 for subsequent microbiological analysis. As previously mentioned (Kim et al. 2005), the use of efficient pre-enrichment and biofilm maturation strategies can thus provide positive benefits to MFC performance. Although the underlying mechanisms by which anodic biofilms become optimized are not yet properly understood, the study of MFC biofilm ecology has been recently attracting much attention (Marsili et al. 2008; Picioreanu et al. 2008; Ramasamy et al. 2008).

The low propionate consumption rates observed (2.5± 1.3% and 6.0±1.1% for the OC- and CC-MFCs, respectively) are likely due to the short hydraulic retention time of the systems (approximately 10 min), which in turn originates from the small reactor volume (5 ml) and the technical difficulty to confidently employ lower flow rates with the peristaltic pumps feeding the media. Such short residence time must have had a strong impact on the composition of the microbial communities developing in the MFCs, affecting the resident's ability to remain in the system, and impacting the capacity of the bacteria resident in the CC-MFCs to use soluble mediators as electron acceptors. A short residence time may translate into a high rate of mediator washout from the system and hence in a higher loss of energy from the populations producing them. We believe that both factors, lower presence of soluble mediators and (or) increased energy expenditure to maintain a sufficient concentration of mediators in the environment, probably led to a shift in the microbial community participating in current production to those able to directly reduce the electrode without the need for soluble mediators and/or those best suited to exist in a biofilm configuration attached to the anode, thus effectively reducing mediators loss. In this sense, the strong influence of residence time on chemical oxygen demand removal and electricity generation has been previously well documented (Moon et al. 2005).

In addition, the degradation of propionate with concomitant electricity production in the CC-MFC does not fully explain the differences observed in propionate consumption in both systems. One probable explanation is that the CC-MFC was able to sustain higher bacterial densities on the anode, and thus the lower than expected electricity production would arise from the increased use of substrate for cell storage, biopolymer production, population growth, etc., all expenditures that reduce coulombic efficiencies. However, the question still remains on how propionate can be metabolized with concomitant electricity generation: (1) direct oxidation of propionate to electricity, (2) breakdown of propionate to H_2 plus acetate and further oxidation of both substrates by EAB, and (3) as in (2) but H_2 following a different fate (e.g., methanogenesis). It has been shown that *G. metallireducens* can directly utilize propionate as an electron donor to reduce metals (Lovley et al. 1993), while *G. sulfurreducens* can utilize acetate and H_2 simultaneously under iron-reducing conditions (Brown et al. 2005). Thus,

both routes 1 and 2 are plausible. However, Jang et al. (2010) observed that under H_2-saturated conditions electricity generation from propionate was reduced, but the coulombic efficiency remained similar compared to N_2-saturated conditions, and hypothesized that propionate was being metabolized through a syntrophic association (as in 3). Further research using co-cultures of propionate oxidizing syntrophs and EAB species may show whether or not H_2 is directly metabolized for electricity generation.

The CEM was fouled by a thick biofilm dominated by two microaerophilic bacteria from the *Achromobacter* (EBL47, 19.2%) and *Azovibrio* (EBL16, 32.7%) genus, sustained by the oxygen diffusing from the aerated cathode chamber. The development of biofilms on the CEMs causes an inefficient transfer of protons from the anode to the cathode, one of the main bottlenecks of MFC systems. Thus, if MFCs carrying CEMs are to be used in the future, the ecology and impact of biofilms growing on the membranes should be better addressed. The composition of the microbial communities present in the liquors seemed to be dominated by sequences arising principally from the anode surface and the *Zoogloea* growing on the proximal section of the influx tubings, but not from sequences derived from the CEM biofilm. The low hydraulic retention time probably accounts for the lack of a specific pelagic community, while the differences in shear force (as a result of the medium flowing through the anode but tangential to the CEM) and relative surface probably determined the lack of dominant populations derived from the CEM biofilm.

In comparison with the OC-MFC anode, the CC-MFC anode was enriched in microorganisms capable of dissimilatory Fe(III) reduction. The higher energy efficiency derived from using the anode as an electron acceptor probably promoted the electrode colonization by such microorganisms, reducing the proportion of anaerobic bacteria (i.e., the observed drop in *Bacteroidetes* spp.) attached to the anode. Moreover, the different *Geobacteraceae* OTUs retrieved, as well as the presence of a *Rikenellaceae* sequence related to an iron-reducing isolate, imply the existence in the CC anode of different ecological niches for the degradation of propionate with concomitant anode reduction.

The results obtained in this study demonstrate that the propionate present in anaerobic wastewater treatment environments can be utilized by EAB with concomitant electricity production and also substantiate the importance of a successful colonization of the anode by EAB and biofilm maturation for the performance of the system. The anodic biofilm environment provided different niches for the degradation of propionate with concomitant electricity production, mostly occupied by species from the *Geobacter* genus. We also describe the community forming the biofilm fouling the CEM; its strong effect on the coulombic efficiencies and power outputs of membrane MFCs is widely accepted, and yet, to our knowledge, its composition has not previously been studied. We believe that the results of this study contribute to the current knowledge of the microbial ecology of MFC systems used for organic waste removal with concomitant electricity production and can be used to improve MFC design and operation.

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