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Substrate removal and electricity generation in a membrane-less microbial fuel cell for biological treatment of wastewater

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HIGHLIGHTS

- A new membrane-less MFC with a biocathode was developed.
- A power density of 30 mW/m^2 and 75.9% substrate degradation efficiency were achieved.
- Pyrosequencing identified Bacteroidia as electron donors in the anode.
- Coulombic efficiencies varied from 19.8% to 58.1%.

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ABSTRACT

Microbial fuel cells have gained popularity in recent years due to its promise in converting organic wastewater into renewable electrical energy. In this study, a membrane-less MFC with a biocathode was developed to evaluate its performance in electricity generation while simultaneously treating wastewater. The MFC fed with a continuous flow of 2 g/day acetate produced a power density of 30 mW/m^2 and current density of 245 mA/m^2. A substrate degradation efficiency (SDE) of 75.9% was achieved with 48.7% attributed to the anaerobic process and 27.2% to the aerobic process. Sequencing analysis of the microbial consortia using 16S rDNA pryosequencing showed the predominance of Bacteroidia in the anode after one month of operation, while the microbial community in the cathode chamber was dominated by Gamma-proteobacteria and Beta-proteobacteria. Coulombic efficiencies varied from 19.8% to 58.1% using different acetate concentrations, indicating power density can be further improved through the accumulation of electron-transferring bacteria.

1. Introduction

Microbial fuel cells (MFCs) have attracted attention for their capability to produce renewable energy from the treatment of organic wastewater (Pant et al., 2012). In an MFC, organic substrates are biologically oxidized by anaerobic bacteria at the anode. The electrons generated during the microbial oxidation reaction are transferred to the anodic electrode and subsequently conducted through an external circuit to the cathode, while protons migrate from the anode chamber to the cathode chamber (Lovley, 2006). On the cathode, the protons combine with electrons and oxygen to form water. The flow of electrons and the positive potential differences between the electrodes give rise to the generation of electrical power (Zhang et al., 2008).

Conventional MFCs consist of biological anodes and abiotic cathodes. The abiotic cathode usually requires a catholyte (e.g., hexacynoferrate or acidic permanganate) to achieve high electron transfer (You et al., 2006) or a metal catalyst (e.g., platinum, pyrolyzed iron (II) phthalocyanine (FePc) or cobalt tetramethylphenylporphyrin (CoTMPP)) with O_2 or air as the cathodic electron acceptor (Cheng et al., 2006; Zhao et al., 2005). However, a catalyst that deactivates with time requires continuous replacement and metal catalysts are easily poisoned by components in the substrate solution and need to be constantly regenerated (Zhao et al., 2006), leading to increased costs and decreased operational sustainability. These systems are therefore impractical and unsustainable for the long-term operation. Such challenges can be overcome by biocathodes, which use microorganisms to assist cathodic reactions (He and Angenent, 2006). Biocathodes have the potential for sustainable operations, but so far few studies have investigated them (Zhang et al., 2008; Huang et al., 2012; Zhuang et al., 2012).

The use of a chemical catholyte or metal catalyst requires the physical separation of the anode and cathode chambers by an ion

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exchange membrane (IEM) to prevent substrate diffusion from the anode to the cathode that leads to a rapid deactivation of the cathode and deterioration of MFC performance (Tartakovsky and Guiot, 2006). IEM inhibits substrate diffusion but permits proton migration from the anode to the cathode. Yet, the application of IEM results in a high internal resistance (Cheng and Liu, 2006) and a retarded transfer of protons from the anode to the cathode, which leads to pH splitting and thus lowers the system stability and bioelectrochemical performance (Kim et al., 2007; Rozendal et al., 2006). Therefore, there exists a counterbalance between higher proton migration and lower substrate diffusion. Another problem for the use of an IEM is biofouling. Membranes used over a period of 50 days were fouled and the biofilm on the membrane caused adverse effects on mass transport through the membrane (Chae et al., 2008). Here again, the use of an IEM increases the overall internal resistance and the overall cost of the MFC.

A membrane-less MFC that uses aerobic microorganisms as a cathodic catalyst was designed and tested in this study. The design, by introducing a unidirectional flow, improves protons and substrate transport from the anode to cathode while reduces oxygen diffusion from the cathode to anode (Fig. 1a). Anolyte mixing is promoted by continuously feeding influent from the bottom of the anode chamber. The organic carbon in medium functions as an electron donor in the metabolic process resulting in the breakdown of the substrate to CO_2 and water in concurrence with the electron generation as a by-product. The unidirectional flow of the remaining organic substrates from the anode to cathode allows further degradation of organic carbon in the cathode chamber by aerobic microbial processes to accomplish the second step of biological oxygen demand (BOD) removal. The vertical stack of two chambers combined with unidirectional flow also prevents the oxygen downward-diffusion. This study demonstrates electricity generation from substrate degradation in the membrane-less MFC with a biocathode and reports the substrate degradation efficiency (SDE), the bacterial morphologies and community composition in each chamber.

2. Methods

2.1. MFC design and construction

The MFC was consisted of two cylindrical polysulfone chambers (Nalgene Co., New York USA) with different volumes (Fig. 1b). The cathode chamber (600 mL total volume) was stacked on top of the anode chamber (800 mL total volume) and the two chambers were separated by a rigid porous plastic spacer, allowing solution to pass from the anode to cathode chamber in a unidirectional flow. For an efficient accumulation of the electrogenesis microflora, ~40 pieces of small carbon felts (~1 cm × 1 cm × 0.6 cm) were filled into the anode chamber as biofilm growth supporters. A graphite rod of 1.27 cm in diameter and 7.62 cm in length (32.9 cm^2 surface area) was used as anode to collect electrons. Cathode was made of a roll-up sheet of carbon felt (15 cm × 3 cm × 0.6 cm). The distance separating anode and cathode electrodes was 4.8 cm. The anode and the cathode were connected by an external copper wire.

2.2. MFC operation

The anode and cathode chambers were inoculated with digested sludge and activated sludge, respectively, collected from a local wastewater treatment plant (Irvine, CA, USA). Synthetic wastewater medium containing 2 g/L acetate as a carbon source (Cao et al., 2009) was pumped into the anode using a peristaltic pump (Barnant Co., lL, USA) at a flow rate of 1 L/day. Synthetic medium contained the following constituents per liter of deionized water: 4.4 g KH_2PO_4; 3.4 g $K_2HPO_4{\cdot}3H_2O$; 1.5 g NH_4Cl; 0.1 g $MgCl_2{\cdot}6H_2O$; 0.1 g $CaCl_2{\cdot}2H_2O$; 0.1 g KCl; 0.1 g $MgSO_4{\cdot}7H_2O$; 0.005 g $MnCl_2{\cdot}4H_2O$ and 0.001 g $NaMoO_4{\cdot}2H_2O$. The effluent from the anode flowed unidirectionally into the cathode by pumping. Air was bubbled into the cathode continuously to provide a dissolved oxygen concentration of 6–7 mg/L. All the experiments were conducted at least in duplicates and at room temperature (24 °C) and atmospheric pressure. To evaluate the influence of the organic nutrient on the performance of MFC, two feeding regimes were tested. The first regime pumped synthetic medium at an acetate-loading rate of 2 g/day at 12-h-on and 12-h-off intervals. The second feeding regime pumped nutrient medium continuously at the same loading rate into the anode and subsequently flow through to the cathode.

2.3. MFC performance, substrate degradation, and microbial colonization

The voltage (V) in the MFC was monitored continuously at 2 min intervals using a multimeter with a data acquisition system (Sinometer instruments, Shenzhen, China). The current (I), power (P), current density (CD), power density (PD) and coulombic efficiency (CE) were calculated as previously described (Liu et al., 2005). PD and CD were normalized to the anode surface area (32.9 cm^2). The polarization and power density curves were obtained by changing external circuit resistances. The dissolved oxygen and pH were measured using a bench top pH meter (Accumet Instruments, Vernon Hills, IL, USA).

Acetate feeding concentration was varied between 1–3 g/L at a constant flow rate of 1 L/day in order to determine SDE and CE as a

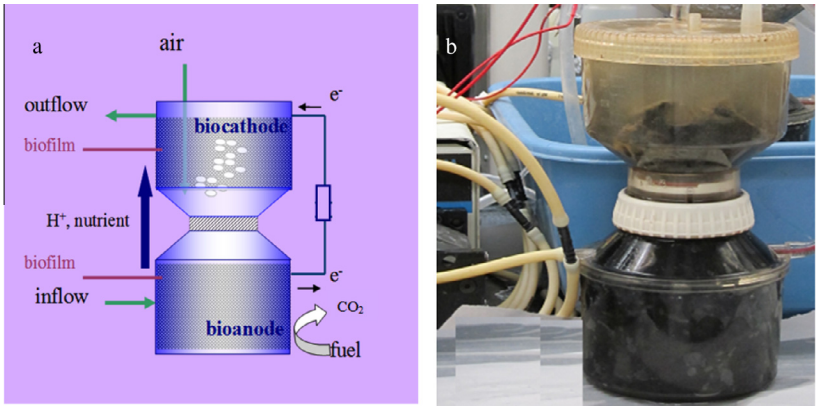

Fig. 1. Schematic (a) and picture (b) of the lab-scale membrane-less MFC.

function of substrate concentration. Substrate degradation was monitored by analyzing chemical oxygen demand (COD) in fluid samples retrieved from both anode and cathode under different initial acetate feeding concentrations (1–3 g/L) in a continuous-fed mode. SDE (%) was computed using:

$$SDE = (COD_i - COD_o)/COD_i \times 100,$$

where COD_i is the influent COD and COD_o is the effluent COD. COD and volatile suspended solids (VSS) analyses were performed according to the Standard Methods (APHA, 1995).

Microbial colonization on the surface of carbon felts was observed using scanning electron microscopy (SEM) (FEI/PHILIPS Co., USA). Briefly, the felts were collected from the chamber after certain period of operation. The bacteria on the felts were fixed with osmium tetroxide in buffer solution of 0.1 M sodium cacodylate, followed by rinsing and dehydration in water/ethanol solutions. Samples were then coated with Au/Pt before SEM observation. Images were captured by a SC1000 ORIUS™ charge-coupled device (CCD) camera.

2.4. Bacterial community phylogenetic analysis

To compare the bacterial communities in anode and cathode, six samples from MFC were collected for 16S rDNA pyrosequencing: the initially inoculated sludge samples from the anode (A1) and the cathode (C1); the mixture of suspended microbial community and biofilm community attached to carbon felt after 1 month of MFC operation (A2 from the anode and C2 from the cathode) and after 4 months of MFC operation (A3 from the anode and C3 from the cathode).

Total genomic DNA was extracted from all samples using PowerSoil DNA Kit (Mo Bio Lab, Carlsbad, CA). The bacterial 16S rDNA PCR and sequencing was performed using primers targeting the variable region V1–V3 for all six samples (Sun et al., 2011). For A1–A3, archaeal 16S rDNA PCR and sequencing were also performed using 340wF and 806R primers (Sun et al., 2011). Tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed by R&T Laboratory using Roche 454 FLX instrument with Titanium reagents (Roche, NJ, USA). The sequences from pyrosequencing were analyzed using QIIME (Quantitative Insights Into Microbial Ecology) Pipeline (Knight et al., 2007). Low-quality sequences (<25) and sequences <200 bp were removed. Sequences that were similar at or above 97% were clustered and defined as an Operational Taxonomic Unit (OTU) for generating rarefaction curves and for calculating the richness and diversity indices. Representative sequences from each OTU were phylogenetically assigned to taxonomic classifications obtained from RDP classifier (Wang et al., 2007). The Shannon-Weiner index (H) was adopted to evaluate the species diversity from each sample using: $H = -\sum p_i \ln p_i$, where p_i is the proportional relative abundance of the ith species (Magurran et al., 1988).

3. Results and discussion

3.1. MFC startup and operation

The MFC electrical voltage output observed using two different feeding regimes is shown in Fig. 2. Under periodic feeding regime, the maximum voltage reached 248 mV after 3 h startup period. The voltage dropped sharply after 20 h of operation to near zero at 22 h. The similar pattern was observed in the second cycle of experiment suggesting exhaustion of nutrients in the chambers during the feeding-off period. Under the continuous nutrient feeding regime, the voltage increased gradually and stabilized at a voltage output of 548 ± 9 mV after 6 days of operation. The voltage of 595 ± 23 mV was maintained for over 3 months of operation with little variation in voltage output and current (data not shown). These experiments showed that the feeding regime of nutrients had an important influence on the output voltage of the MFC. Continuously feeding nutrients would maintain continuous solution transportation from anode to cathode, which improved protons transfer in the same route.

The pH measurements over time during MFC operation showed the anolyte pH decreased gradually from 7.83 to 7.11 and the catholyte pH increased steadily from 7.04 to 8.24 over 15 days of MFC operation while the pH of the nutrient inflow was consistent at 7.50 ± 0.12. Oxygen reduction in the cathodic chamber explains the increase in pH, while anaerobic microbial activity and accumulation of protons results in a decreasing pH in the anodic chamber. The trends of pH change in both chambers are in accordance with active bio-electricity generation in MFC. The relative stabilization of pH in this study suggests the dramatic pH split observed in previous studies during operation of a MFC with a membrane (Rozendal et al., 2006; Jacobson et al., 2011) can be significantly relieved.

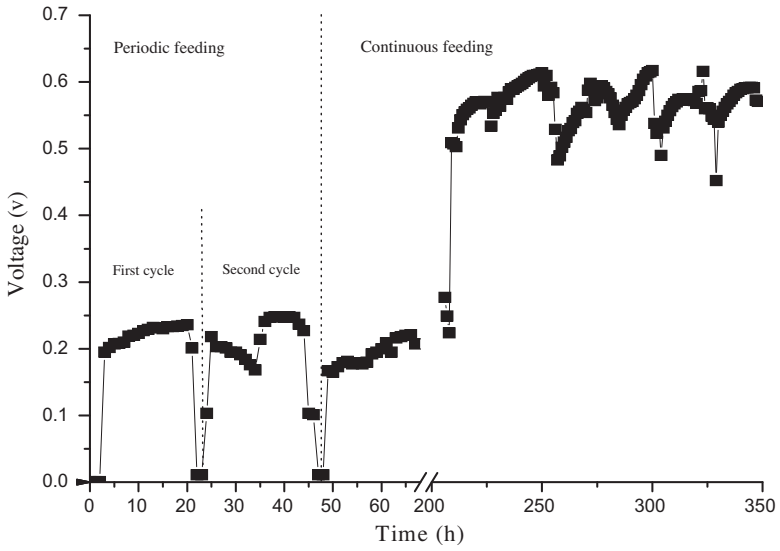

Fig. 2. Voltage generated using membrane-less MFC using different organic nutrient feeding operations (periodic and continuous nutrient loading).

In the traditional MFC system, proton transport through the membrane is slower than the proton production rate in the anode chamber and the proton consumption rate in the cathode chamber (Rozendal et al., 2006). The membrane-less design enables a unidirectional flow, which improves proton transport through convection. In addition, eliminating the IEM greatly decreases the cost for MFC construction since the costly IEMs (such as Nafion) contributes a significant portion of capital investment. Thus, this cost-effective feature increases the feasibility for practical application (linked with wastewater treatment) (Pant et al., 2011).

A key concern of membrane-less MFC system is oxygen in the cathode may diffuse to the anode, affecting electrochemical activity of the anodic microorganisms and thus reducing the electrochemical potential or cell voltage (Liu et al., 2005). The experimental measurements in this study showed that dissolved oxygen concentration in the cathode solution, where air bubbles were blown in, was around 6–7 mg/L. In a liquid solution, the oxygen diffusivity (D_{O2}) can be evaluated using a hydrodynamic model, which is developed by assuming that the resistance of solute molecule movement is caused by the viscous force, similar to a particle movement in a viscous fluid. In a dilute liquid, the approach yields the well-known Stokes–Einstein equation: $D_{O_2} = k_B T/6\pi r\mu$, where k_B is the Boltzmann's constant, T the temperature, r the radius of the oxygen molecule, and μ the electrolyte viscosity. Using the properties of liquid water, the oxygen diffusivity is around 10^{-9} m^2/s. The Peclet number of oxygen transport (Pe_{O_2}) is defined to compare the convection and diffusion effects (Wang et al., 2010), as follow: $Pe_{O_2} = uL/D_{O_2}$, where u and L represent the average flow velocity and characteristic length. In the MFC, L is $\sim$ 0.01–0.1 m, and u is $\sim 10^{-5}$ m/s, yielding Pe_{O_2} of $\sim$100–1000. Thus, convection dominates oxygen transport. That is, the oxygen diffusion towards the anode is efficiently depressed by the unidirectional flow. This computational result was confirmed by experimental measurements indicating the oxygen content was nearly zero in the anode. These results, together with a recent report indicating no reduction in the performance of the electrodes when a gas porous activated charcoal was used in place of a conventional proton exchange membrane in an air–cathode MFC (Pant et al., 2010a,b), indicate anaerobic activities at anode are not significantly impacted by the air–cathode. Similarly, in the electrolyte, the proton transport from the anode to the cathode is driven by diffusion, migration and convection, and its resistance can be a limiting factor in MFC performance. Similar to oxygen transport, the convective flux of protons is much stronger than the diffusive one, improving proton transport towards the cathode and hence reducing its transport resistance.

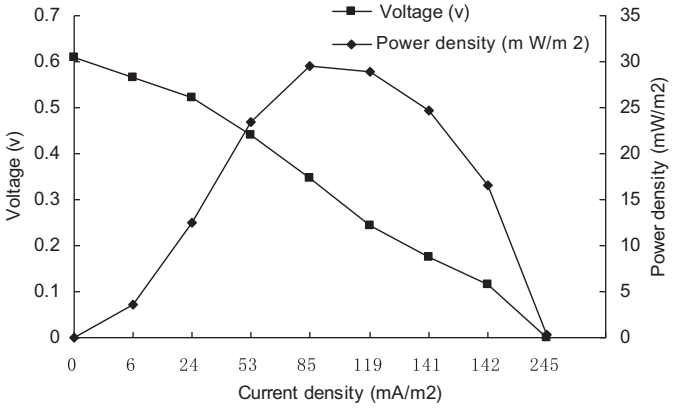

Fig. 3. Polarization and power density curves of MFC.

3.2. Power generation and substrate degradation

By varying the circuit resistance from 0 to 40 kΩ, the polarization curve of the MFC was obtained and a maximum power density of 30 mW/m^2 at 85.49 mA/m^2 (1.9 kΩ) was obtained using the concentration of 2 g/L acetate as the substrate. The current density reached as high as 245 mA/m^2 (30 Ω) (Fig. 3). Based on the method employed by Liang et al. (2007), the internal resistance of the MFC was equal to the external resistance, (1.9 kΩ) when the maximum power density was obtained. Although it is difficult to directly compare the power output with other MFC performances in the literature due to different operating conditions, surface area and type of electrodes, and different microorganisms involved (Pant et al., 2010a,b), further improvement of power density is possible in the MFC performance. Long-term enrichment and cultivation of bacteria in MFCs could lead to accumulation of electron-transferring bacteria and mediators and subsequently increased power production during MFC operation (Sevda et al., 2013). Recently, several research groups have characterized electrochemically active anode communities to potentially improve MFC performance by addressing microbial constraints. The potential of large increases in power production using bacteria that produce their own mediators was demonstrated (Rabaey et al., 2004).

In order to further investigate the relationship between substrate degradation and electrical output, the SDE and the CE were measured and calculated at three different acetate-feeding concentrations in continuous-fed mode (Table 1). Total substrate removal was 62.8–75.9%, including 28.4–48.7% of substrate degradation in the anode chamber and 27.2–34.4% of that removed in the cathode chamber at different initial substrate concentrations. The CE decreased from 58.1% to 19.8% as the acetate concentration increased from 1 to 3 g/L, while the voltage of the experiments (1 kΩ external resistance) was <0.250 V for all tests.

Given the easily degradable nature of acetate and both aerobic and anaerobic treatment processes in the MFC, the overall COD removal efficiency of 62.8–75.9% was low. This is likely due to the overloading of acetate at 1–3 g/day (concentration of 1–3 g/L at 1 L/day flow rate). The microorganisms present in the MFC might be insufficient to degrade the substrate efficiently at such high acetate-loading rate. However, the observation of overall substrate removal demonstrated that the MFC was linked with the anaerobic–aerobic wastewater treatment processes. Further studies are required to explore maximum substrate loading rate capacity for the MFC. The observed relationship between SDE in the anode chamber and CE during the MFC operation at different acetate concentrations could be attributed to the substrate consumption by the microorganisms that do not contribute to electron generation, such as methanogens, instead of the electron-transferring bacteria; or the low electron transfer efficiency from solution to anode due to the absent/low level of electron mediators in the anolyte.

It should also be noted that SDE and CE presented in this study maybe at their maximal efficiency with the current design because acetate was used as substrate to benchmark the new MFC components, reactor designs, and operational conditions (Chae et al., 2009; Pant et al., 2010a,b). Electricity generation in MFCs fed with food or domestic wastewaters is considered as a substrate with a

Table 1
Substrate degradation efficiency and coulombic efficiency at different initial acetate concentration.

Initial acetate concentration (g/L)	Total SDE (%)	SDE in anode (%)	SDE in cathode (%)	CE (%)
1	73.2 ± 2.0	39.9 ± 1.3	33.2 ± 3.2	58.1 ± 0.8
2	75.9 ± 0.4	48.7 ± 2.5	27.2 ± 2.1	52.4 ± 1.9
3	62.8 ± 2.1	28.4 ± 1.7	34.4 ± 0.6	19.8 ± 1.4

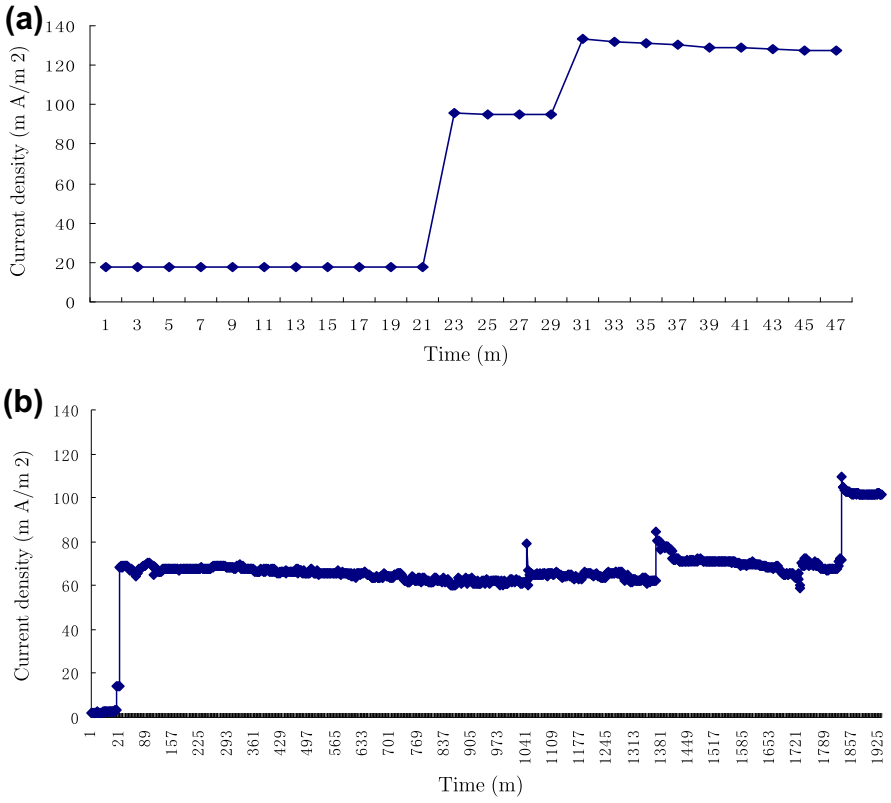

Fig. 4. Current generation in MFC after replacing the existing catholyte (a) and anolyte (b) with fresh medium to remove suspended bacterial biomass. The current is measured using1000 Ω external resistance.

Table 2
The number of sequences and community diversity from each sample.

Samples	Descriptions	Identified sequences	Classes	Genera	OUT_S	Shannon-Weiner index
A1	Bac-16SrDNA sequences of sample collected from anode before MFC operation	3555	26	101	125	3.19
A2	Bac-16SrDNA sequences of sample collected from anode after 1 month MFC operation	3122	35	157	183	3.49
A3	Bac-16SrDNA sequences of sample collected from anode after 4 months MFC operation	3525	34	130	161	3.13
A1a	Arch-16SrDNA sequences of sample collected from anode before MFC operation	8508	4	8	11	0.31
A2a	Arch-16SrDNA sequences of sample collected from anode after 1 month MFC operation	5638	2	8	20	1.56
A3a	Arch-16SrDNA sequences of sample collected from anode after 4 months MFC operation	3332	3	10	25	2.34
C1	Bac-16SrDNA sequences of sample collected from cathode before MFC operation	3827	28	157	182	3.6
C2	Bac-16SrDNA sequences of sample collected from cathode after 1 month MFC operation	2419	18	86	106	2.91
C3	Bac-16SrDNA sequences of sample collected from cathode after 4 months MFC operation	2840	25	113	139	3.63

relatively lower CE compared to acetate due to its fermentable nature (Lefebvre et al., 2011; Patil et al., 2009). Liu et al. (2009) reported that the MFC based on acetate-induced consortia achieved more than twofold maximum electric power when compared to the MFC based on consortia induced by protein-rich wastewater. Since acetate is the end product of several metabolic pathways for higher order carbon sources, a hydrolysis pretreatment for converting complex organics to simpler compounds might be added as the pretreatment unit before degradation byproducts such as acetate can be fed into the MFC. The pretreatment could result in decreasing the lag time to produce electricity and in achieving higher power densities compared with using actual wastewater directly in MFC.

3.3. Bacterial community in the MFC

3.3.1. Bacterial growth and colonization

The quantity of bacterial catalysts is one of the most critical factors for the performance of MFC. We used carbon felt as the electrode because of its relatively large specific surface area per volume (60.5 m^2/g) in comparison with other materials such as carbon paper (0.3 m^2/g) to improve microbial density. SEM images revealed that both anode and cathode were covered with biofilm (see Supplemental sFig. 1). Long bacterial filaments and rope-like bacterial aggregates were observed on the anode while the biofilm on the cathode appeared less in comparison (sFig. 1). However, suspended biomass in the cathode was over twice that in the anode (VSS was measured as 1942 mg/L in catholyte and 900 mg/L in anolyte).

To understand the role of electrode-attached bacteria and suspended biomass in electricity generation, we replaced the existing catholyte with fresh medium to remove suspended bacterial biomass, which resulted in drop in electrical current from 127 to 20 mA/m^2 at 1000 Ω external resistance. However, the current rapidly returned to the original level after approximately 30 min of operation (Fig. 4a). Removal of suspended bacterial biomass in the anode by replacing the existing anolyte with fresh medium caused current to drop near zero at 1000 Ω external resistance

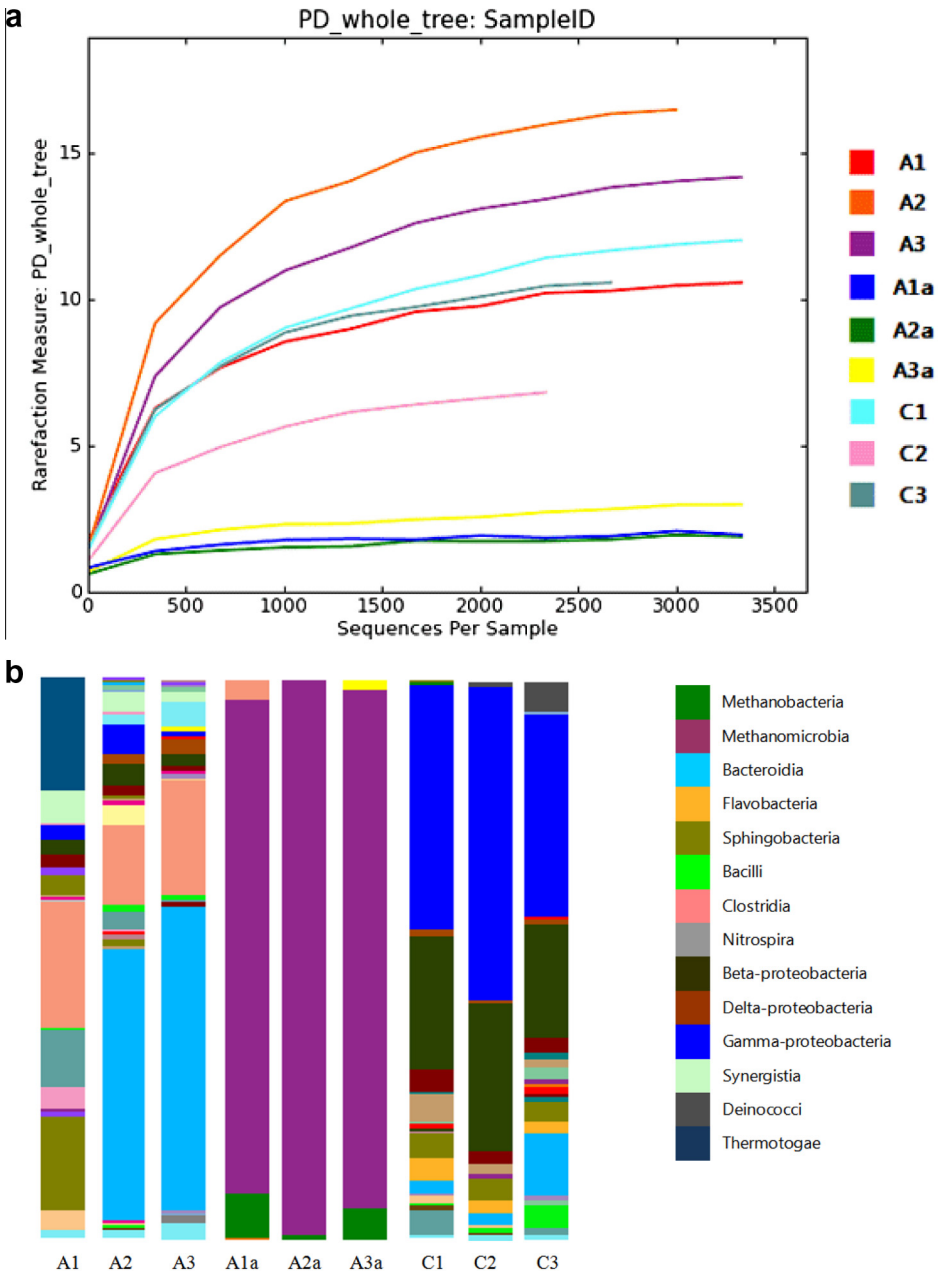

Fig. 5. Bacterial and archaeal community of MFC. (a) Rarefaction curves of phylogenetic diversity. (b) Comparisons of bacterial and archaeal community at different sampling dates in the anode and cathode chambers (class levels).

(Fig. 4b). The current recovered slowly to 70 mA/m^2 after 30 min of operation and was maintained for nearly 30 h before recovering back to the original current density (Fig. 4b). These results imply bacteria directly attached to the cathode had a preferred pathway to utilize electrons directly from the electrode while both attached and suspended bacteria in the anode are important for electricity generation (Behera and Ghangrekar, 2009; Sun et al., 2009). The initial shock in electricity production after exchange of anolyte could be due to the introduction of dissolved oxygen to the anode that sieges the anaerobic microbial activity. Further research is necessary to illustrate the underlying mechanism. Improvement of electron scavenging bacteria colonization on the cathode may significantly increase the power generation.

3.3.2. Community diversity

16S rDNA sequencing of microbial consortia associating with anode and cathode yielded a total of 36,766 qualified sequencing reads (Table 2) that were clustered to a total of 952 OTUs. The diversity of bacterial community in the anode and cathode changed during MFC operation (Table 2). The bacterial diversity in the anode chamber (A1–A3) increased after one month of operation and stabilized at around 130 genera or 34 classes at the end of the 4 months. The Shannon-Weiner index of these three samples (A1–A3) was similar. On the contrary, the bacterial diversity in the cathode chamber (C1–C3) decreased after one month of operation and then stabilized at around 113 genera or 25 classes. Similarly, the Shannon-Weiner index mirrored this pattern. The rarefaction curves (Fig. 5a) indicated that the number of sequences from all samples reached plateau implying sufficient sampling of genetic diversity in each sample. Archaeal 16S rDNA sequencing results showed there was relatively low diversity of archaeal community in the anode. A total of 10 genera were identified at the end of 4-month operation, the highest number of genera identified since the startup of the MFC. The increasing Shannon-Weiner index

in A1a to A3a indicated that although the number of class or genera of archaeal communities was not changed much, species diversity and species richness were developed during the MFC operation. Rarefaction curve again indicated the depth of sequencing was sufficient to cover the diversity of archaeal community in each sample.

3.3.3. Community composition

Clostridia were the most dominant class (22.8%) in the initial inoculation of the anode, followed by Thermotogae (20.1%), Sphingobacteria (16.6%) and Synergistia (5.7%). This composition reflects the bacterial community in the digested sludge where thermophilic, mesophilic anaerobic bacteria are commonly observed (Hernon et al., 2006). During the MFC operation, the bacterial community changed from a thermophilic-anaerobes dominated community to an anaerobic community with diverse metabolic pathways. Bacteroidia accounted for 48.7% of the sequences in the anode after one-month operation and 54.2% after four-month MFC operation (Fig. 5b). In the contrast, relatively similar archaeal compositions were observed in the anode chamber. The archaeal community was dominated by Methanomicrobia and Methanobacteria, suggesting the possibility of methanogenesis pathway in the anode chamber.

The predominance of Bacteroidia in A2 after one month of operation of the MFC suggests that this group may be involved in electric current generation. The predominance of the Bacteroidia was also reported in previous MFC and MEC studies (Kim et al., 2006; Zhang and Angelidaki, 2012), while the observed predominance of Proteobacteria and Geobacter were reported in other previous studies (Zhao et al., 2005; Cheng et al., 2006; Phung et al., 2004), which suggests the bacterial community structure in the anode can be affected by the differences in electron donors, sources of inoculum and the operating conditions in the MFCs. Bacteroidetes tend to be the initial soil bacterial community members that metabolize labile organic matter (Padmanabhan et al., 2003). Therefore, the obvious increases of this group and of the voltage in the MFC within the first month demonstrate that the MFC could be used to enrich a bacterial community that oxidizes organic substrates with concomitant electron transfer to the electrode in a short term. However, the similar bacterial community between A2 and A3 samples and the slow enrichment of the predominant class in the next 3 months indicates that the further enrichment of the predominant class cannot be easily performed under the same operating environment. Further improvements may be achieved by understanding and engineering the microbial ecology at the anode.

Comparing the three samples from the cathode at the different sampling dates (C1–C3), similar bacterial compositions were observed. Gamma-proteobacteria constituted the most abundant class accounting for 36.3–55.9% of the sequences, followed by 20–26.6% Beta-proteobacteria, 1.8–10.8% bacteroidia, 0.1–5.4% Deinococci, 3.6–4.5% Sphingobacteria, 1–4.8% Nitrospira and 2.4–4.2% Flavobacteria (Fig. 5b).

The predominance of Gamma-proteobacteria and Beta-proteobacteria in the cathode was in accordance with results from previous studies on activated sludge (Eschenhagen et al., 2003; Wells et al., 2009). Beta-proteobacteria, including the genera *Nitrosomonas* and *Nitrospira*, and Gamma-proteobacteria are the most well-known ammonia-oxidizing bacteria. Holmes et al. (2004) also found that the microorganisms on the cathode of a sediment MFC participated in ammonia oxidation. These results imply that these ammonia oxidizing bacteria in the MFC may be the electrochemically active bacteria that contribute to electron acceptance from the cathode electrode. However, since suspended biomass played a secondary role in cathode in comparison with the electro-attached bacteria, the dominant electron acceptance bacteria deserve further investigation.

4. Conclusions

The membrane-less unidirectional flow MFC with biocathode provides a proof-of-concept demonstration of a technology to link MFC with the anaerobic–aerobic wastewater treatment that has been adopted in most municipal wastewater treatment plants. The predominance of Bacteroidia in the anode chamber contributed to the current generation after one month of MFC operation, while the ammonia oxidizing bacteria in the cathode chamber contributed to accept electrons from the cathode electrode. Further improvement of power density by engineering the microbial ecology in both chambers and the electricity generation using the actual biodegradable wastewater will be studied in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.03.172.

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