

Electricity Generation Using an Air-Cathode Single Chamber Microbial Fuel Cell in the Presence and Absence of a Proton Exchange Membrane

HONG LIU[†] AND BRUCE E. LOGAN^{*,†,‡}

Department of Civil and Environmental Engineering and The Penn State Hydrogen Energy (H₂E) Center, The Pennsylvania State University, University Park, Pennsylvania 16802

Microbial fuel cells (MFCs) are typically designed as a two-chamber system with the bacteria in the anode chamber separated from the cathode chamber by a polymeric proton exchange membrane (PEM). Most MFCs use aqueous cathodes where water is bubbled with air to provide dissolved oxygen to electrode. To increase energy output and reduce the cost of MFCs, we examined power generation in an air-cathode MFC containing carbon electrodes in the presence and absence of a polymeric proton exchange membrane (PEM). Bacteria present in domestic wastewater were used as the biocatalyst, and glucose and wastewater were tested as substrates. Power density was found to be much greater than typically reported for aqueous-cathode MFCs, reaching a maximum of $262 \pm 10 \text{ mW/m}^2$ ($6.6 \pm 0.3 \text{ mW/L}$; liquid volume) using glucose. Removing the PEM increased the maximum power density to $494 \pm 21 \text{ mW/m}^2$ ($12.5 \pm 0.5 \text{ mW/L}$). Coulombic efficiency was 40–55% with the PEM and 9–12% with the PEM removed, indicating substantial oxygen diffusion into the anode chamber in the absence of the PEM. Power output increased with glucose concentration according to saturation-type kinetics, with a half saturation constant of 79 mg/L with the PEM-MFC and 103 mg/L in the MFC without a PEM (1000 Ω resistor). Similar results on the effect of the PEM on power density were found using wastewater, where $28 \pm 3 \text{ mW/m}^2$ ($0.7 \pm 0.1 \text{ mW/L}$) (28% Coulombic efficiency) was produced with the PEM, and $146 \pm 8 \text{ mW/m}^2$ ($3.7 \pm 0.2 \text{ mW/L}$) (20% Coulombic efficiency) was produced when the PEM was removed. The increase in power output when a PEM was removed was attributed to a higher cathode potential as shown by an increase in the open circuit potential. An analysis based on available anode surface area and maximum bacterial growth rates suggests that mediatorless MFCs may have an upper order-of-magnitude limit in power density of 10^3 mW/m^2 . A cost-effective approach to achieving power densities in this range will likely require systems that do not contain a polymeric PEM in the MFC and systems based on direct oxygen transfer to a carbon cathode.

* Corresponding author phone: (814)863-7908; fax: (814)863-7304; e-mail: blogan@psu.edu.

[†] Department of Civil and Environmental Engineering.

[‡] The Penn State Hydrogen Energy (H₂E) Center.

Introduction

Bacteria can be used to catalyze the conversion of organic matter into electricity (1–7). Fuel cells that use bacteria are loosely classified here as two different types: biofuel cells that generate electricity from the addition of artificial electron shuttles (mediators) (8–12) and microbial fuel cells (MFCs) that do not require the addition of a mediator (5, 7, 13–15). It has recently been shown that certain metal-reducing bacteria, belonging primarily to the family *Geobacteraceae*, can directly transfer electrons to electrodes using electrochemically active redox enzymes such as cytochromes on their outer membrane (16–18). These so-called mediatorless MFCs are considered to have more commercial application potential than biofuel cells because the mediators used in biofuel cells are expensive and toxic to the microorganisms (7). MFCs typically produce power at a density of less than 50 mW/m^2 (normalized to anode projected surface area) (7, 13, 19). In a MFC, two electrodes (anode and cathode) are each placed in water in two chambers joined by a proton exchange membrane (PEM). The main disadvantage of a two-chamber MFC is that the solution cathode must be aerated to provide oxygen to the cathode. It is known that the power output of an MFC can be improved by increasing the efficiency of the cathode. For example, power is increased by adding ferricyanide (20) to the cathode chamber. It is possible, however, to design a MFC that does not require that the cathode be placed in water. In hydrogen fuel cells, the cathode is bonded directly to the PEM so that oxygen in the air can directly react at the electrode (21). This technique was successfully used to produce electricity from wastewater in a single chamber MFC by Liu et al. (15). Park and Zeikus (20) produced a maximum of 788 mW/m^2 using a unique system with a Mn^{4+} graphite anode and a direct-air Fe^{3+} graphite cathode (20).

Because the power output of MFCs is low relative to other types of fuel cells, reducing their cost is essential if power generation using this technology is to be an economical method of energy production. Most studies have used relatively expensive solid graphite electrodes (7, 19), but graphite-felt (14) and carbon cloth (15) can also be used. The use of air-driven cathodes can reduce MFC costs because passive oxygen transfer to the cathode using air does not require energy intensive air sparging of the water. Finally, PEMs such as Nafion are quite expensive. We wondered if this material was essential for power production in an MFC. We therefore designed and constructed a carbon-cloth, air-cathode fuel cell to try to increase power density to levels not previously achieved with aqueous cathode systems. To study the effect of the PEM on power production, we compared power density for glucose and wastewater feeds with the system in the presence and absence of a polymeric PEM.

Materials and Methods

Air-Cathode MFC Construction. The MFC consisted of an anode and cathode placed on opposite sides in a plastic (Plexiglas) cylindrical chamber 4 cm long by 3 cm in diameter (empty bed volume of 28 mL; anode surface area per volume of $25 \text{ m}^2/\text{m}^3$) (Figure 1). The anode electrodes were made of Toray carbon paper (without wet proofing; E-Tek) and did not contain a catalyst. The carbon electrode/PEM cathode (CE-PEM) was manufactured by bonding the PEM directly onto a flexible carbon-cloth electrode containing 0.5 mg/cm^2 of Pt catalyst (E-Tek). The PEM (Nafion 117, Dupont) was sequentially boiled in H_2O_2 (30%), deionized water, $0.5 \text{ M H}_2\text{SO}_4$, and deionized water (each time for 1 h). The PEM

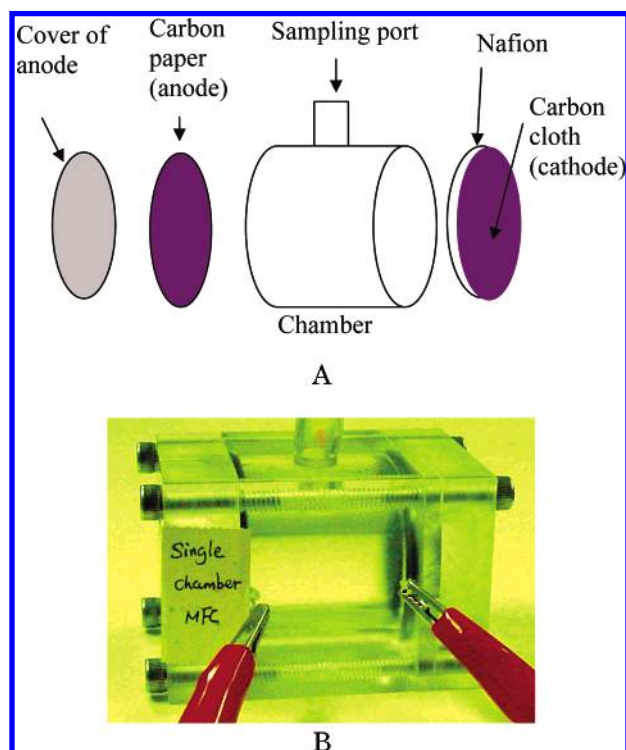


FIGURE 1. (A) Schematic and (B) laboratory-scale prototype of single chamber MFC used to generate electricity from wastewater and glucose.

was then hot-pressed directly onto the cathode by heating it to 140 °C at 1780 kPa for 3 min. The cathode used in the absence of the PEM was a more rigid carbon paper containing 0.35 mg/cm² of Pt (E-Tek). Platinum wire was used to connect the circuit (1000 Ω resistor except when stated otherwise).

Microorganisms and Medium. Bacteria present in wastewater were used to inoculate the MFCs as wastewater bacteria have been shown to be suitable biocatalysts for electricity production (15, 20). Domestic wastewater (primary clarifier effluent) was obtained from the Pennsylvania State University Wastewater Treatment Plant and used as both the inoculum and the substrate. The wastewater had a pH ranging from 7.3 to 7.6 and a chemical oxygen demand (COD) of 200–300 mg/L. A glucose (170–1200 mg/L) medium was also used that contained the following (per liter): NH₄Cl, 310 mg; KCl, 130 mg; NaH₂PO₄·H₂O, 4.97 g; Na₂HPO₄·H₂O, 2.75 g; and a mineral (12.5 mL) and vitamin (12.5 mL) as reported by Lovley and Phillips (22).

Experimental Conditions. The anode chamber was repeatedly filled with wastewater until bacteria in the wastewater colonized the electrodes and produced electricity. The chamber was refilled when the voltage decreased to less than 50 mV (1000 Ω resistor). For experiments with glucose at different concentrations, the anode was refilled each time with fresh medium containing 0–1200 mg/L of glucose (as indicated). Experiments were conducted in a constant temperature room (30 °C) in duplicate.

Calculations. Voltage was measured using a multimeter with a data acquisition system (2700, Keithly) and converted to power density, P (W/m²), according to $P = IV/A$, where I (A) is the current, V (V) the voltage, and A (m²) the cross-sectional area (projected) of the anode. The Coulombic efficiency was calculated as $E_c = C_p/C_{Ti} \times 100\%$, where C_p (C) is the total Coulombs calculated by integrating the current over time. C_{Ti} (C) is the theoretical amount of Coulombs that can be produced from either wastewater ($i = w$) or glucose ($i = g$), calculated as

$$C_{Ti} = \frac{Fb_iS_i v}{M_i} \quad (1)$$

where F is Faraday's constant (98 485 C/mol of electrons), b_i is the number of mol of electrons produced per mol of substrate ($b_w = 4$, $b_g = 24$), S_i (g/L) the substrate concentration, v (L) the liquid volume, and M_i the molecular weight of the substrate ($M_w = 32$, COD basis; $M_g = 180$).

The amount of oxygen that diffuses through a Nafion membrane was estimated based on the measurements of oxygen diffusion in hydrogen fuel cells by others (23, 24). The rate of oxygen diffusion through a membrane (W) can be calculated as

$$W = -DA \frac{dC}{dx} \cong -DA \frac{\Delta C}{\delta_m} \quad (2)$$

where D is the diffusion coefficient of oxygen in the membrane ($1-6 \times 10^{-6}$ cm²/s; refs 23 and 24); δ_m the PEM thickness (190 μm; ref 24); A the cross-sectional area (7.1 cm²); and ΔC the concentration difference defined as the maximum assuming saturation of oxygen in the membrane ($3.7-6.1 \times 10^{-7}$ mol/cm³) (23, 24) and no oxygen in the inner section of membrane.

The oxygen flux across the cathode/membrane, or cathode alone, was calculated by measuring dissolved oxygen accumulation in the anode chamber over time using a nonconsumptive fiber optic oxygen probe (Foxy oxygen probe, SF2000, Ocean Optics, Dunedin, FL) in glucose-free medium. The reactor was stirred during these tests using a small magnetic stir bar inserted into the anode chamber. In a completely mixed container, the dissolved oxygen concentration can be expressed as a function of time, t , and the flux of the chemical across electrode surface as

$$v \frac{dC}{dt} = JA \quad (3)$$

Using the previous approximation of the flux, we have

$$\frac{dC}{dt} = \frac{-DA(C - C_w)}{v\delta_m} \quad (4)$$

where C is the bulk oxygen concentration, C_w the concentration at the CE/PEM or CE (assumed to be the saturation concentration of oxygen in water, or 2.65×10^{-4} mol/L), v the volume of the anode chamber (28 mL), and δ_m the thickness of the cathode/membrane (190 μm, assuming the PEM provided the main resistance to mass transfer for the combined CE/PEM). In the absence of the carbon electrode, we report only the effective mass transport coefficient, $k = D/\delta_m$. Integrating and solving for the time needed to reach one-half the oxygen saturation value ($C_w/2$), we have

$$t = \frac{\ln(0.5) v \delta_m}{-DA} \quad (5)$$

Voltage was modeled as a function of substrate concentration (S) using a Monod-type equation as

$$V = \frac{V_{max} S}{K_s + S} \quad (6)$$

where V_{max} the maximum voltage and K_s (V) the half-saturation constant were determined using the Matlab 6.0 with Optimization Toolbox 2.1 (25).

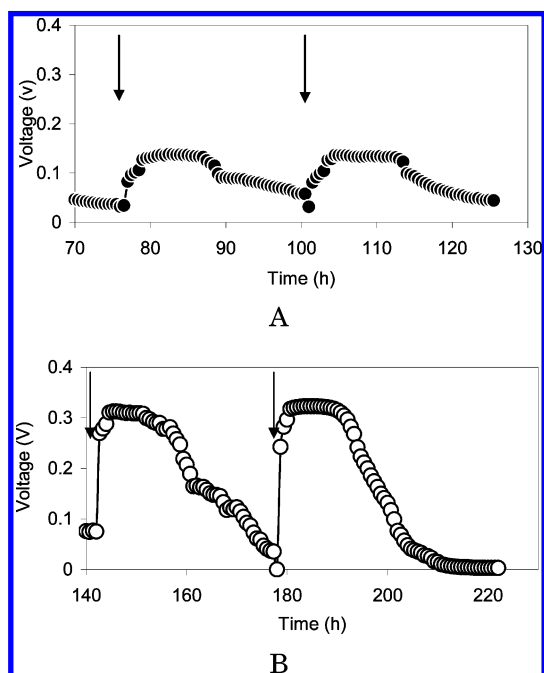


FIGURE 2. Electricity generation by MFCs during startup using domestic wastewater: (A) system with carbon electrode coated with PEM (CE/PEM) and (B) system with only the carbon electrode (CE) and no PEM (1000 Ω resistor). (Arrows show the replacement of wastewater.)

Analytics. Glucose was measured using the phenol-sulfuric acid method (26). COD was measured according to standard methods (27). Acetone, fatty acids (acetate, propionate, butyrate), and alcohols (methanol, ethanol, *n*-propanol, and *n*-butanol) were analyzed using a gas chromatograph (Agilent, 6890) equipped with a flame ionization detector and a 30 m \times 0.32 mm \times 0.5 μ m DB-FFAP fused-silica capillary column. Samples were filtered through a 0.2 μ m pore diameter membrane and acidified using formic acid (0.65 M) before GC analysis. The temperature of the GC column was started at 60 $^{\circ}$ C, increased at 20 $^{\circ}$ C/min to 120 $^{\circ}$ C, and then 30 $^{\circ}$ C/min to a final temperature of 240 $^{\circ}$ C for another 3 min. The temperatures of injector and detector were both 250 $^{\circ}$ C. Helium was used as the carrier gas at a constant pressure of 103 kPa.

Bacteria on the electrodes were examined using a scanning electron microscope (SEM) (JSM 5400, JEOL). Samples were fixed overnight in 2.5% paraformaldehyde and 1.5% glutaraldehyde in buffer (0.1 M cacodylate, pH 7.4) at 4 $^{\circ}$ C, washed three times in buffer, dehydrated stepwise in a graded series of water/ ethanol solutions (25, 50, 70, 85, 95, 100%), and then dried (critical-point carbon dioxide). Samples were then sputter coated with Au/Pt prior to SEM observation.

Results

Electricity Generated from Wastewater. Stable power generation was obtained after only two sequential transfers of domestic wastewater into the carbon-cathode MFC containing a PEM. After 78 h, replacing the wastewater produced for a ca. 10 h period a stable voltage of 0.14 ± 0.01 mV (Figure 2A). Thereafter, the cell voltage slowly declined to 0.05 V over the next 12 h. This pattern of voltage output was reproducible when fresh wastewater was added into the fuel cell even though no precautions were taken to maintain anaerobic conditions during liquid replacement. Stable power generation using the MFC without a PEM bonded to the carbon cathode took longer, requiring four transfers of fresh wastewater into the anode compartment. However, after 140 h operation, a consistent maximum

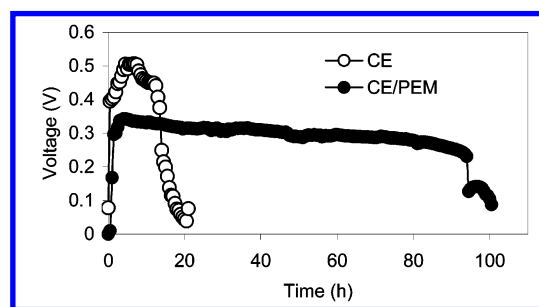


FIGURE 3. Voltage generation from glucose (600 mg/L) in the MFC with carbon electrode coated with PEM (CE/PEM) and the MFC with only the carbon electrode (CE) and no PEM (1000 Ω resistor).

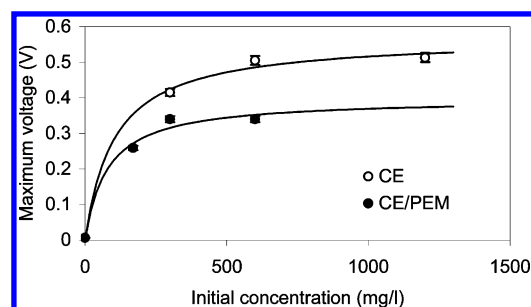


FIGURE 4. Maximum voltage generation as a function of glucose concentration (Error bars \times 1SD based on two cells with experiments conducted in duplicate.)

voltage could be achieved for ca. 10 h of 0.32 ± 0.01 V, declining to <0.10 V after 20 h (Figure 2B). On the basis of the circuit resistance (1000 Ω), the MFC without the PEM generated 146 ± 8 mW/m 2 (3.7 ± 0.2 mW/L; liquid volume), a power density 5.2 times that achieved with the MFC containing a PEM (28 ± 3 mW/m 2 , 0.7 ± 0.1 mW/L).

Electricity Generated from Glucose. Electricity was immediately generated from glucose (600 mg/L; 1000 Ω) using the MFCs inoculated with bacteria in wastewater (Figure 3). In the carbon electrode MFC containing a PEM (CE-PEM), a maximum of 0.35 V (173 mW/m 2) was obtained, with the power slowly dropping to 0.25 V over the next 95 h, and then sharply decreasing over the next 5 h. In the MFC without a PEM (CE), a maximum of 0.52 V (381 mW/m 2) was generated with the voltage rapidly decreasing to low levels (<0.044 V) within 20 h. Voltage output appeared to follow saturation kinetics as a function of glucose concentration, with a maximum of 0.57 V and half-saturation constant of $K_s = 103$ mg/L for the MFC without a PEM ($R^2 = 0.997$) and 0.40 V and $K_s = 79$ mg/L with a PEM ($R^2 = 0.988$) (Figure 4).

Power Density as a Function of Circuit Resistance. To determine what load (resistance) would produce the maximum power density, the circuit resistance was varied from 150 to 5000 Ω with glucose as the substrate. The maximum power from the MFC without a PEM reached 494 ± 21 mW/m 2 (12.5 ± 0.5 mW/L) producing a current of 0.86 mA (465 Ω) (Figure 5). The MFC with a PEM generated of 262 ± 10 mW/m 2 (6.6 ± 0.3 mW/L) with a current of 0.92 mA (218 Ω).

Coulombic Efficiency. About 55% of COD was removed using the MFC with a PEM, and 75% was removed using the MFC without a PEM. The Coulombic efficiencies using wastewater were 28% for the MFC with a PEM and 20% for the MFC lacking a PEM. For the glucose-fed MFC, over 98% of glucose was removed. Acetone, several fatty acids (acetate, butyrate, propionate), and alcohols (ethanol, propanol, butanol) were detected during glucose degradation, but only small amounts of acetate (<6 mg/L) remained in solution when the voltage had dropped to <0.050 mV. Neglecting these components, the Coulombic efficiency of the glucose-

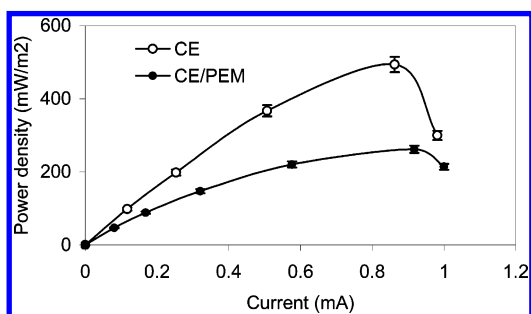


FIGURE 5. Power density generated as a function of current (Error bars $\times 1$ SD based on triplicate measurements of the circuit potential.)

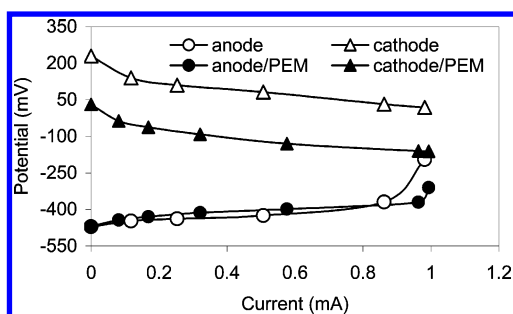


FIGURE 6. Anode and cathode potential (vs Ag/AgCl reference electrode, 195 mV, vs NHE) as a function of current.

fed MFCs (98% glucose removal) was 40–55% for the MFC with a PEM and 9–12% for the MFC without the PEM.

Oxygen Transfer. On the basis of diffusion coefficients reported by Basura et al. (24) and Parthasarathy et al. (23), and assuming that oxygen transfer is limited in the CE/PEM cell only by the resistance (and thickness) of the PEM, it is estimated that the rate of oxygen diffusion into the anode chamber was in the range of 0.016–0.16 mg/h. For the MFC with the PEM, we measured a half-life of 200 ± 3 min, resulting in an estimate of $D = 4.4 \times 10^{-6}$ cm²/s, a value within the range of $1\text{--}6 \times 10^{-6}$ cm²/s reported by others (23, 24). For the MFC with a PEM, $k = 2.3 \times 10^{-6}$ cm²/s, resulting in the potential for a maximum oxygen flux of 0.05 mg/h. For the MFC without PEM, we measured a half-life of 53 ± 13 min or about one-fourth of that in the presence of the PEM producing $k = 8.6 \times 10^{-6}$ cm²/s. In the absence of the PEM, the oxygen flux could reach 0.187 mg/h, or a rate 2.7 times larger than that in the MFC with the CE/PEM.

Electrode Potential. To further examine the effect of the PEM on power generation, we measured the open circuit potential (OCP) and the working potentials of the electrodes as a function of current (up to 1 mA) by varying the circuit load. The OCP and working potentials of the anodes were found to be quite similar, despite the presence or absence of a PEM (−0.469 and −0.470 V, respectively) (Figure 6) based on an Ag/AgCl reference electrode (0.195 V corrected to a normal hydrogen electrode; NHE). The working potential of the anodes increased slightly with current up to ca. 0.86 mA. At a higher current, the potential was unstable consistent with findings by others (6).

There were significant differences in the OCP and working potentials of the cathode in the presence and absence of a PEM (Figure 6). The OCP when the PEM was present was −0.031 V (0.226 V NHE). Without a PEM, the OCP was 0.230 V (0.425 V NHE). This difference in voltage (0.177 ± 0.012 V) was apparent over a range of working potentials up to a current of nearly 1 mA. This voltage difference could be a result of several different factors. First, by removing the PEM, we likely decreased the internal resistance in the system producing less resistance for proton transfer from the anode

to the cathode. Less internal resistance could result in the observed increase in potential. Second, it is also possible that heating the PEM and cathode to bond them together caused carboxylation and degeneration of the catalyst on the carbon cloth cathode, producing a less efficient cathode. Third, the catalyst content on cathode with PEM (0.5 mg Pt/cm²) was higher than that without the PEM (0.35 mg Pt/cm²). However, the greater Pt content on the system with a PEM should have increased the efficiency of the system, not decreased the voltage. Thus, we consider the decrease in internal resistance to be the most likely explanation for the increased voltage in the system lacking a PEM.

Further improvements in power density can probably still be achieved with changes in the cathode construction. Under standard conditions, the cathode potential E (NHE basis) for air can be calculated based on Nernst equation (28) using

$$E = 1.2288 + 0.0148 \log p - 0.05915 \text{ pH} \quad (7)$$

as 0.804 V (assuming a partial pressure of oxygen of $p = 0.2$ units and a pH = 7). This is much larger than the potentials measured here of 0.425 V (no PEM) or 0.226 V (PEM), indicating further improvements in cathode performance are possible.

The OCPs measured here of −0.274 and −0.275 V (NHE basis) suggest electrons are carried away from a respiratory enzyme at some point between that of NAD and cytochrome *c*. The redox potential for NAD⁺/NADH is −0.320 V (pH 7.0) (29), while that of cytochrome *c* is −0.190 V (30). Others have reported a lower potential of 0.170 V, using a pure culture of *Geobacter sulfurreducens*, which suggested that electron transfer from the cell occurred at cytochrome *c* (7).

Biofilms on Electrodes. Micrographs taken of the electrodes show that a biofilm developed on both electrodes (Figure 7). Bacteria growing on the anode appeared to be somewhat uniform in morphology, and many bacteria grew as chains. In contrast, the biofilm that grew on the cathode was much more heterogeneous.

Discussion

By using an air-cathode MFC without a PEM, it was possible to produce as much as 146 mW/m² with domestic wastewater (primary clarifier effluent). This is approximately an order of magnitude larger than power densities reported for other complex materials such as anaerobic sediments (16–28 mW/m²), a high-starch content wastewater (19–20 mW/m²), or domestic wastewaters (24 mW/m²) (Table 1). The high power density achieved here is due to both the use of the air cathode and the removal of the PEM. Sediment fuel cells that lack a PEM have not achieved large power densities, likely as a result of the inefficiency of the aqueous cathode. In a similar air-cathode MFC using graphite rod anodes and a PEM, 24 mW/m² was produced (15), a value comparable to the 28 mW/m² produced in the MFC here with carbon electrodes and a PEM. Thus, it appears both the absence of a PEM and the use of the air cathode resulted in the high power densities with the mixed substrates.

When glucose was used as a substrate by bacteria for electricity generation, up to 494 mW/m² was produced in the air-cathode MFC in the absence of the PEM. In general, the use of defined substrates such as lactate, acetate, and glucose has resulted in power densities an order of magnitude or more lower than that achieved here (i.e. in the range of 0.3–49 mW/m² (MFCs with a PEM) (Table 1)). A power density of 262 mW/m² was achieved here with the air-cathode MFC when a PEM was used, suggesting that the air cathode alone was responsible for a substantial increase in power output as compared to other studies using aqueous cathodes. Park and Zeikus (20) achieved a power density of 788 mW/m² only by using a type of air-cathode system based on Fe³⁺

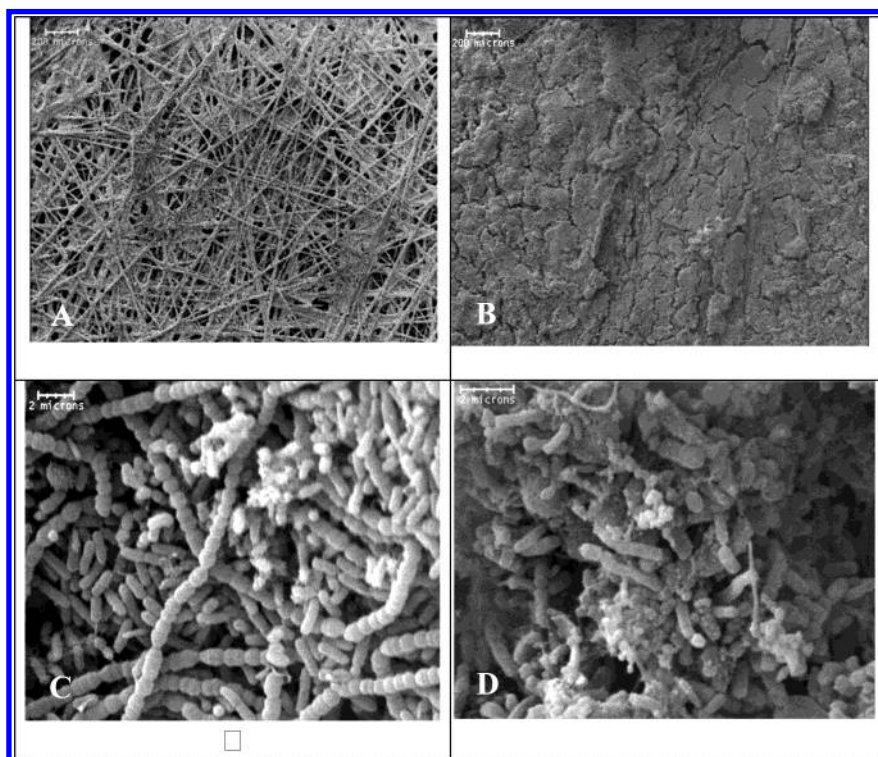


FIGURE 7. SEM images of (A) plain anode, (B) anode with biofilm developed, (C) bacteria on the anode surface, and (D) bacteria on the cathode surface.

TABLE 1. Power Generation Rates Reported in the Literature versus Those Obtained in This Study

substrate	description	power (mW/m ²)	ref
complex	anaerobic sediments	16	4
	starch wastewater	19	34
	starch wastewater	20	6
	domestic wastewater	24	15
	anaerobic sediments	28	19
	domestic wastewater – CE-PEM	28	this study
	domestic wastewater – CE (no PEM)	146	this study
defined	lactate	0.6	5
	lactate	10	35
	lactate	15	13
	lactate, peptone, and yeast extract	788	20
	acetate (salt bridge)	0.3	31
	acetate	14	36
	acetate	40	31
	acetate	49	13
	glucose	33	14
	glucose – CE-PEM	262	this study
	glucose – CE (no PEM)	494	this study
	glucose	3600	32

bonded to a porous ceramic material and a Mn^{4+} anode containing a bound mediator; they also did not use a polymer membrane, and their iron electrode was an air-driven cathode. Thus, it appears that common elements of their design and ours that achieved high power output were the use of air-cathodes and the lack of a polymeric PEM. Both of these studies also used wastewater bacteria as the biocatalyst as opposed to pure cultures used in several other studies (5, 7, 31).

The highest power density for a MFC published to date is 3600 mW/m² (32). In that study, potassium hexacyanoferrate was used to optimize the performance of the cathode. Even with this advantage, it seems likely that mediators were involved in producing a power density this large. We estimated the maximum power that can be produced in a mediator-less MFC by assuming a monolayer of bacteria on

an electrode surface, a high growth rate of $m = 8.3 \text{ days}^{-1}$ (12 doublings per day), a cell yield of $Y, 3.6 \times 10^{13} \text{ cell/mol}$ of acetate (22), and that cells ($0.5 \times 1.5 \text{ mm}$) pack perfectly onto the surface leaving no open area achieving a cell density of $X = 1.33 \times 10^{12}/\text{m}^2$. The maximum power density generated can then be calculated based on the open circuit potential of 0.8 V as

$$P = IV = \left(\frac{\mu X}{Y} b_e F \right) V \quad (8)$$

where $b_e, 8 \text{ e}^-/\text{mol}$ of acetate. Using these values, we calculate a power density of 2200 mW/m². A calculation based on values obtained with glucose produced a similar result. This estimate could be reduced if it is recognized that bacteria cannot completely cover 100% of a surface and that cells

that die or other bacteria that do not produce electricity can occupy space on the surface. Alternatively, this calculated power level could be increased by including additional surface provided by the anode material that results from its surface roughness (i.e., the actual vs projected surface area). Thus, it seems reasonable that an upper limit for power generation limited by bacterial growth on the anode is on the order of 10^3 mW/m² in a mediatorless MFC. The power density of 3600 mW/m² achieved by Rabaey et al. (32) is certainly within the range of this order of magnitude estimate of power generation, but we consider it likely that the bacteria produced their own mediators to achieve this high level of power generation. The presence of mediators would allow more than a monolayer of bacteria to participate in power generation and could result in substantially increased power densities as compared to those achieved by others. Even if mediators were produced by bacteria in their MFC, we would still classify their bacterial fuel cell as a mediatorless MFC based on the fact that they did not add exogenous mediators into the fuel cell. It is also possible that mediators have been produced in other studies when mixed cultures or even pure have been used, as bacteria such as *S. putrefaciens* are known to produce their own mediators (33).

Implications of Using MFCs That Do Not Contain a Polymeric PEM. The main disadvantage of using a MFC without a PEM is the potential for the loss of substrate due to aerobic oxidation by bacteria in the anode chamber. Even with polymeric membranes such as Nafion, there can be significant oxygen diffusion into the anode chamber. It is estimated, based on oxygen flux measurements here, that oxygen diffusion into the anode chamber was in the range of 0.05 mg/h (CE/PEM). With the PEM removed, this increased to 0.187 mg/h (CE). In the presence of the PEM, values obtained here for oxygen diffusivities through the PEM are consistent with oxygen diffusion results for hydrogen fuel cells. In the absence of the PEM, however, the rate of oxygen transfer into the anode chamber substantially increased. This oxygen was likely consumed by bacteria growing on the cathode as it was observed in SEM images that a biofilm developed on the surface of the cathode (anode chamber side) (Figure 7). We were unable to measure dissolved oxygen in the anode chamber with power generation using either glucose or wastewater. In addition, when the cathode containing Pt was replaced with a plain carbon electrode, there was little power generation (<10%) even after sufficient time for a bacterial biofilm to grow (data not shown). This indicates that the Pt, and not the bacteria, was the critical catalyst at the cathode. Thus, it is likely that the bacteria growing on the cathode were consuming oxygen diffusing through the membrane while oxidizing substrate in the anode chamber.

The Coulombic efficiencies obtained here using the air cathode MFCs are generally lower than those reported by others using aqueous cathode MFCs. We found that the Coulombic efficiency was 40–55% in the glucose-fed MFC in the presence of a PEM and that the efficiency was only 9–12% when the PEM was removed. These values are substantially lower than 83 and 97% reported by others using pure cultures (7, 14) and aqueous cathodes. However, Coulombic efficiencies in other studies have varied widely, with values of 89% (32), 12% (6), 3–12% (15), and 0.04% (34). Park and Zeikus (20) did not report Coulombic efficiencies for their air-cathode system, and it is not possible to measure efficiencies for marine sediment MFCs when no substrate is added to the system.

The most likely reason for a low Coulombic efficiency is the loss of substrate due to diffusion of oxygen through the membrane, although other factors may contribute to reduced efficiencies as well. Oxygen diffusion through the Nafion membrane at a rate measured here of 0.05 mg/L could

account for the aerobic loss of 28% of the glucose added at a concentration of 600 mg/L when the cell is operated for 100 h. If we consider this loss of substrate to oxygen, the unaccounted portion of electrons based on our Coulombic efficiency calculation would decrease from 45 to only 17%. Another reason for a wide range of Coulombic efficiency could be different resistors used in the various studies. An increase of Coulombic efficiency was observed when the circuit resistance decreased (6). In the study by Rabaey et al. (32), a Coulombic efficiency of 89% was observed, but the resistance of 100 Ω is lower than that used in many other studies. In studies using wastewater or defined media, it is also possible that there is a substantial loss of electrons to alternate electron acceptors such as sulfate and nitrate. Only a small amount of sulfate (0.35 mmol/L) was added to defined media used here, a level that would account for only 4% of substrate losses. Bacterial yields, production of mediators, and other factors could affect Coulombic efficiencies as well.

Despite of the relatively low Coulombic efficiencies of an air-cathode design that lacks a polymeric PEM, there still may be a role for MFCs in wastewater treatment for several reasons. First, the organic matter in a wastewater is free; thus, its loss to aerobic processes is not intrinsically a problem as such a loss accomplishes wastewater treatment (15). Second, it is likely that losses due to oxygen diffusion can be reduced either through improvements in coatings on the side of the cathode exposed to the chamber or perhaps through continued biofilm development. The reactors used here were all tested over a relatively short period of time (days), but even after this period, there was substantial development of a biofilm on the carbon cathode (Figure 7). Third, the system described here is not optimized with respect to anode and cathode relative surface areas and other factors that might change or reduce the surface area needed for the cathode.

It has been shown here that carbon-cloth electrodes can be used instead of graphite electrodes and that the PEM can be eliminated while increasing power generation, two factors that reduce the cost of making MFCs. Pt coatings were used on our carbon cathode electrodes, but it may be possible to reduce levels of Pt needed, or even eliminate Pt on electrodes by using other catalysts in the future. With continued improvements in fuel cell catalysts and materials, it may therefore be possible to increase power generation rates in MFCs and lower their cost. For wastewater treatment, even the use of passive oxygen transfer into a reactor can help to reduce plant operation costs in comparison to aerated treatment systems such as activated sludge reactors. Thus, the combination of electricity production and reduced aeration costs must both be included in a final analysis of the cost-effectiveness of using MFCs in wastewater treatment plants.

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