



Pre-acclimation of a wastewater inoculum to cellulose in an aqueous–cathode MEC improves power generation in air–cathode MFCs

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ABSTRACT

Cellulose has been used in two-chamber microbial fuel cells (MFCs), but power densities were low. Higher power densities can be achieved in air–cathode MFCs using an inoculum from a two-chamber, aqueous–cathode microbial electrolysis cell (MEC). Air–cathode MFCs with this inoculum produced maximum power densities of 1070 mW m⁻² (cathode surface area) in single-chamber and 880 mW m⁻² in two-chamber MFCs. Coulombic efficiencies ranged from 25% to 50%, and COD removals were 50–70% based on total cellulose removals of 60–80%. Decreasing the reactor volume from 26 to 14 mL (while maintaining constant electrode spacing) decreased power output by 66% (from 526 to 180 mW m⁻²) due to a reduction in total mass of cellulose added. These results demonstrate that air–cathode MFCs can produce high power densities with cellulose following proper acclimation of the inoculum, and that organic loading rates are important for maximizing power densities from particulate substrates.

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1. Introduction

The development of improved types of microbial fuel cells (MFCs) has proceeded at a rapid pace in recent years due to their potential to produce renewable energy from various types of waste biomass (Logan, 2009). Most studies have been conducted with specific types of chemicals, such as glucose and various carbohydrates, volatile fatty acids, amino acids and proteins (Pant et al., 2009). More complex sources of organic matter sources such as domestic wastewater have also been used in MFCs (Ahn and Logan, 2009), but power densities are generally lower than those obtained with single substrates (Pant et al., 2009). Many wastewaters and waste biomass consist of a high percentage of particulate biomass, and this form of substrate requires longer reaction times in MFCs due to the need to hydrolyze the substrate into smaller molecules that can be directly taken into the cell.

Cellulose is the most abundant biopolymer in the world and is considered as to be an ideal source of organic matter for renewable energy production. However, power densities produced in MFCs directly from cellulose have been very low, and successful power generation has relied on using specialized cultures in two-chamber MFCs or in sediment MFCs. Power was produced by *Enterobacter cloacae* from cellulose, but the power density was very low (4.9 mW m⁻²) (Rezaei et al., 2009). A co-culture of *Clostridium cellulolyticum* that can ferment cellulose to hydrogen and volatile

fatty acids and solvents, and the exoelectrogen *Geobacter sulfurreducens*, were used in co-culture to produce low power densities (<60 mW m⁻²) from cellulose (type MN301) in a two-chamber MFC (Ren et al., 2007). Similarly low power densities (55 mW m⁻²) were achieved using mixed cultures with a rumen inoculum (Rismani-Yazdi et al., 2007). Low power densities were initially achieved by Rezaei et al. (2008) using a wastewater inoculum in a two-chamber MFC with a cation exchange membrane, but they found that reproducible cycles of power could not be achieved over multiple cycles without the addition of cellulase enzymes. A power density of 29–62 mW m⁻² was produced with a sediment MFC using anodes wrapped around cellulose particles that were placed in a sediment (Rezaei et al., 2007).

The primary reason for the low power densities reported in previous studies is the high internal resistance of two-chamber MFCs. There have been no reports of direct power generation in air–cathode MFCs with cellulose as a sole substrate, although cellulose-containing wastewaters were shown to produce 627 mW m⁻² power when phosphate buffer was added to the wastewater (Huang and Logan, 2008). In preliminary studies in our laboratory, we found that we were unable to generate appreciable power from cellulose using a wastewater inoculum in an air–cathode MFC. However, we had shown current generation in a microbial electrolysis cell (MEC) with cellulose (Cheng and Logan, 2007). We wondered if the relatively high rate of oxygen diffusion into an air–cathode MFC could have limited development of the anode biofilm for power generation, as oxygen can diffuse into the reactor. In an MEC, the reactor is sealed off from air. To avoid oxygen during biofilm development

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we examined inoculum development in an MEC that was then used in two different types of air–cathode MFCs.

2. Methods

2.1. Media and enrichment conditions in a two-chamber MEC

Domestic wastewater collected from the primary clarifier effluent at the Pennsylvania State University Wastewater Treatment Plant and used as the original inoculum for a two-chamber (H-type) MEC (Logan et al., 2006) containing an anion exchange membrane (AMI-7001, Membranes International). The anode was carbon paper (20 × 60 mm, E-TEK), and the cathode (2 cm²) was carbon cloth with a Pt catalyst (0.5 mg Pt/cm²). The anode chamber was filled with a mixture of cellulose (1.5 g/L; Sigmacell[®] Cellulose, Type 20 with Microcrystalline Structure, Sigma), wastewater, and a phosphate buffered nutrient medium (PBS; 50/50 v/v). The cathode contained only PBS (conductivity of 11.2 mS/cm), consisting of (per L): 4.9 g NaH₂PO₄·H₂O, 9.15 g Na₂HPO₄·H₂O, 0.31 g NH₄Cl, 0.13 g KCl; and 12.5 mL of a mineral solution and 5 mL of a vitamin solution (Cheng et al., 2009). Both electrodes were connected to the external circuit using a titanium wire (0.68 mm in diameter, Alfa Aesar, Ward Hill, MA, US). A voltage of 0.3 V was added to the MEC using a power source (model 3645A; Circuit Specialists, Inc.). After 10 days, when hydrogen gas production was achieved in the cathode chamber, the anode biofilm containing both the cellulolytic bacteria and the extracellular electron transfer bacteria was scraped off and added to PBS, and stored at 4 °C for later use.

2.2. MFC construction and operation

Two types of air–cathode MFCs were used: single-chamber MFCs lacking a membrane, and two-chamber MFCs. The two-chamber MFC contained a 4 cm long anode (28 mL) and a 2 cm long cathode chamber (14 mL), with both chambers 3 cm in diameter (4 + 2 cm MFC) with an AEM (Kim et al., 2007). Except as noted below (for the variable-volume MFCs), the single-chamber system had the same two blocks joined together into a single chamber (6 cm MFC; 42 mL) in order to maintain the same electrode spacing (3 cm), but no membrane was used. Anodes were ammonia gas treated graphite fiber brushes (25 mm diameter × 25 mm length; fiber type PANEX 33 160 K, ZOLTEK), and cathodes (7 cm²) contained Pt (0.5 mg/cm²) and four polytetrafluoroethylene (PTFE) diffusion layers (Cheng et al., 2006).

MFC anode chambers were inoculated with the stored bacterial suspension from the MECs, cellulose (2 g/L), and PBS, while the cathodes contained only PBS. The MFCs were operated with a fixed 1 k Ω external resistance in fed-batch mode. Solutions were replaced when the voltage decreased to <20 mV. To maintain suspensions of bacteria in the anode capable of cellulose hydrolysis, only 10% of the anode solution was replaced in subsequent cycles using 20 g/L of cellulose in fresh PBS that was deoxygenated by nitrogen gas sparging. The cathode chamber (two-chamber MFC) was completely refilled with PBS. MFCs were operated in a constant temperature room (30 °C) in duplicate.

In additional experiments, the size of the MFC was reduced to examine the effect of the total mass of cellulose added to the system, but with the concentration of cellulose unchanged in order to avoid potentially large pH changes. To vary the mass added, MFCs were used that had three volumes of 14, 20, and 26 mL, produced by using reactor chamber lengths of 2, 3, and 4 cm. Electrode spacing was kept constant by placing the edge of the anode ~1 cm from the cathode. The same size anodes described above were used for the 3 and 4 cm long MFCs, but the brush size had to be reduced in the 2-cm long MFC (14 mm in diameter and 25 mm in length) in order to avoid short circuiting by the brush touching the cath-

ode. Other results (unpublished data, our laboratory) have demonstrated that a reduction in mass of fibers used for the anode in the amounts examined here do not affect power production. Reducing the anode volume resulted in a decrease in total mass loaded into the anode chamber (from 52 to 28 mg). This reduced the rate that soluble organics could be supplied to the anode from the hydrolysis of the cellulose.

2.3. Bacterial community and phylogenetic analysis

Total genomic DNA extracted from the anode biofilm, gene cloning, and 16S rRNA gene sequencing for community analysis were conducted as previously described (Kiely et al., 2010) using a PowerSoil DNA isolation kit (MO BIO Laboratories). A total of 96 clones were selected and sequenced from each anode sample. Plasmids were sequenced with the M13R primer using an ABI 3730XL DNA sequencer (Applied Biosystems). The nucleotide collection (nr/nt) of the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>) was searched using the BLASTn algorithm to analyze the sequences. 16S rRNA fragments of clones representing distinct phylotypes were plotted against the total number of clones. Sampling coverage, rarefaction curves, and Shannon diversity values were calculated as previously described (Kiely et al., 2010).

2.4. Calculations and measurements

Voltage (E) across the external resistor (1 k Ω , except as noted) in the MFC circuit was measured at 20 min intervals using a data acquisition system (2700, Keithley Instrument, OH) connected to a personal computer. Current, power and coulombic efficiency were calculated as previously described (Logan et al., 2006), with the current density and power density normalized by the projected surface area of the cathode. Polarization and power density curves were obtained using a different external resistance (1000–50 Ω) for 20 min. The ohmic resistance was measured by electrochemical impedance spectroscopy (Cheng et al., 2006).

The concentration of cellulose was measured using a colorimetric method (Ren et al., 2007). Fatty acid concentrations were determined using a high performance liquid chromatograph (HPLC; Waters 486, Milford, Mass.) equipped with a HPX-87H column (Biorad, Hercules, Calif.) (Kiely et al., 2010). The COD of the solution at the end of the cycle was measured using standard methods (APHA, 1998).

3. Results and discussion

3.1. Startup of MFCs

The MFCs required only a few fed-batch cycles (~5–10 days) to demonstrate reproducible power cycles after inoculation with anode solution from the MECs. Once acclimated for stable voltage production, the voltage curves showed three distinct phases: an initial, gradual build up in voltage; a maximum voltage that was sustained over several days; and then a gradual decline in voltage (Fig. 1a). This gradual increase and plateau in the voltage curve likely reflects the release and accumulation of cellulose degradation products into solution and accumulation of these substrates by bacteria to levels sufficient to consistently produce high voltages in the system.

3.2. Maximum power densities in single- and two-chamber MFCs

Based on polarization data obtained with stable and maximum voltages during fed-batch cycles, the maximum power density was

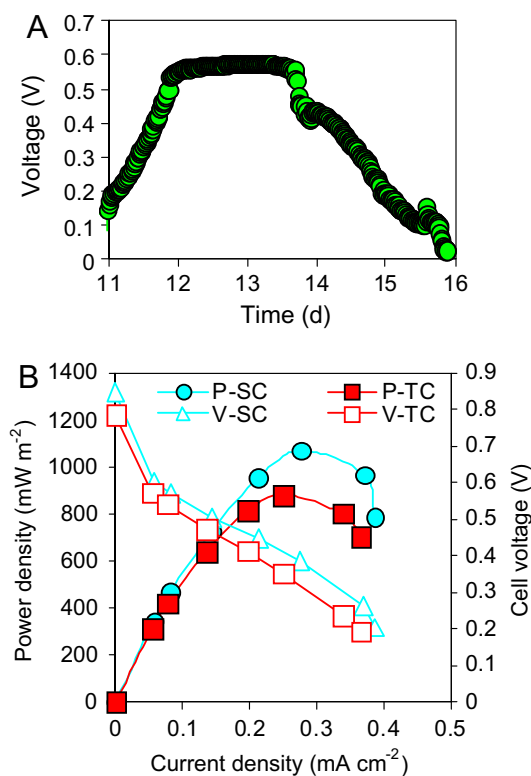


Fig. 1. (A) Example of a single cycle of voltage generation in a single-chamber (SC; 6 cm) MFC at a fixed resistance. (B) Voltage and power density as a function of current density obtained by varying the external circuit resistance in single-chamber and two-chamber (TC; 4 + 2 cm) MFCs.

$1070 \pm 15 \text{ mW m}^{-2}$ for the 6 cm single-chamber MFCs. This high power density was possible due to the development of a stable, cellulose-degrading culture in a low internal resistance MFC. The use of an AEM, which can reduce oxygen transfer from the cathode to the anode, produced a higher ohmic resistance of 40Ω , compared to 33Ω for the single chamber lacking a membrane. The two-chamber MFC with the AEM produced a lower maximum power density of $880 \pm 20 \text{ mW m}^{-2}$ (4 + 2 cm MFC) (Fig. 1b).

The pH changes over the fed-batch cycle were similar for the single- and two-chamber MFCs, despite the presence of the membrane in the two-chamber system. The final pH was 6.54 for the single-chamber MFC, and 6.48 in the anode chamber of the two-chamber MFC. Both types of MFCs had similar CEs, COD removals, and cellulose degradation efficiency. For example, CEs were $25 \pm 4\%$ (single-chamber) and $27 \pm 3\%$ (two-chamber), and COD removals were $75 \pm 10\%$ (single-chamber) and $68 \pm 8\%$ (two-chamber) ($1 \text{ k}\Omega$ resistor). Cellulose degradation efficiencies were $80 \pm 10\%$ (single-chamber) and $76 \pm 10\%$ (two-chamber). Decreasing external resistance from 1000 to 50Ω increased the CEs to $\sim 50\%$, but decreased the COD removal to $\sim 50\%$ and the cellulose degradation efficiency to 60% .

3.3. Power generation using single-chamber MFCs with reduced volumes

Reducing the volume of reactor from 26 to 14 mL decreased the total mass of cellulose added into the reactor (from 52 to 28 mg). This reduction in total mass shortened the voltage generation cycle, and in the case of the 14 mL reactor, substantially reduced the maximum peak in voltage. The power density normalized to cathode surface area decreased from 526 to 180 mW m^{-2} (for a $1 \text{ k}\Omega$ resistor) with volume (Fig. 2). The volumetric power density

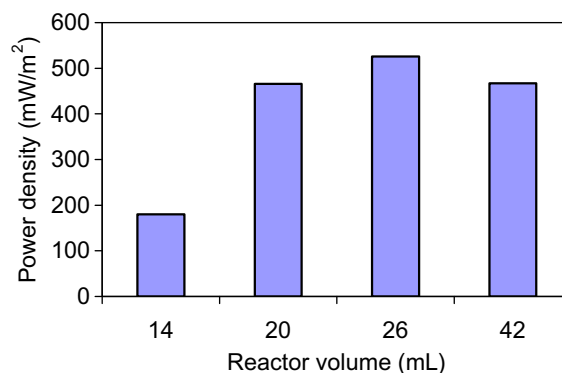


Fig. 2. Power generation as a function of reactor volume in the single-chamber MFCs. Note that the electrode spacing was constant ($\sim 1 \text{ cm}$) for the 14–26 mL. The 42 mL reactor, shown for comparison, had a larger electrode spacing (3 cm).

increased by 14% from 14 to 16 W m^{-3} when the reactor volume was decreased from 26 to 20 mL. However, a further reduction in volume to 14 mL decreased the maximum power by 44% to 9 W m^{-3} (Fig. 2). These changes in power production were not due electrochemical changes in the system, but to biological changes. The electrode spacing (between cathode surface and brush fiber surface closed to the cathode) was kept at $\sim 1 \text{ cm}$ for all three reactors, and the ohmic resistance was unchanged ($13 \pm 1 \Omega$).

This single-chamber maximum power density ($1070 \pm 15 \text{ mW m}^{-2}$) is over 18 times higher than that previously reported with two-chamber MFCs using either co-cultures or mixed cultures and cellulose (Ren et al., 2007; Rismani-Yazdi et al., 2007), and 1.7 times as large as that obtained using a single-chamber MFC with a cellulose wastewater (Huang and Logan, 2008). The reasons for the larger power density of the single-chamber MFC compared to the two-chamber MFCs were lower ohmic resistance and the mass of cellulose loaded into the system. Maximum power density is well known to be a function of internal resistance (Logan et al., 2006). The single-chamber MFC used here had 18% less ohmic resistance than the two-chamber system, which was the main reason the power density increased by $\sim 10\%$. For the same reason, power densities produced using a single-chamber MFC by Huang and Logan (2008) were larger than those of Ren et al. (2007) and Rismani-Yazdi et al. (2007) due to the lower internal resistance of the single-chamber MFC design.

Although the electrode spacing was the same in the single and two-chamber MFCs, they also differed in the total mass of cellulose loaded into the system. The anode chamber of the two-chamber MFC (26 mL) had less total mass of cellulose than the single-chamber system (42 mL). When we systematically looked at total mass of cellulose in the anode chamber of the MFC by changing the volume of the anode chamber from 14 to 28 mL (but not changing the cellulose concentration), power density decreased with reactor volume and thus mass of cellulose. Therefore, we infer that the larger mass of cellulose contributed to a greater release of cellulose hydrolysis products, resulting in higher power densities. This effect of total mass of cellulose was seen for the smallest MFC of 14 mL not only by a shorter cycle time (due to less available substrate), but also by a lack of a plateau in the voltage generated, indicating there was insufficient substrate to reach and sustain a higher power density (Fig. 2).

A key component of the high power densities achieved here was the way the inoculum was developed, and how bacteria were maintained in the system. We first developed the anode in an MEC, avoiding the possibility of oxygen leaking into the system and interfering with the development of anaerobic microorganisms. This approach to avoid oxygen intrusion could also be

accomplished using ferricyanide or other chemical catholytes as done by others (Ren et al., 2007). In addition, we maintained the population of bacteria the system more effectively by replacing only a portion of the anode solution each cycle, rather than the complete anode solution as done by others where power from the cellulolytic consortium was lost over time (Rezaei et al., 2008). Power generation using sediment MFCs has also been shown with particulate substrates such as chitin and cellulose (Rezaei et al., 2007), and in these systems the bacteria that grow on these substrates remain in the sediment in close proximity to the anode, likely aiding sustained power generation.

3.4. 16S rRNA community characterization of anode biofilm

16S rRNA community analysis identified the anode biofilm to be dominated by clones with significant similarity (<97%) to members of the *Clostridiaceae* family (Fig 3). Clones representing *Clostridium thermocellum* (62%) represented the largest subset of the total population. *Geobacter sulfurreducens* isolates were also indicated to comprise a significant proportion of the microbial community (17% of clones). The number of clones used to analyze this anode community was significant based on a Goods coverage of 89%. Community diversity was quite low, with a Shannon Diversity index of 1.05.

Analysis of the microbial community in the MFC showed that the culture was dominated by *C. thermocellum*. This microbe is well known to be useful for lignocellulose degradation, and has previously been used in a two-stage process for maximizing hydrogen production in an MEC (Lalauette et al., 2009). This microorganism has also been used in co-cultures for cellulose degradation, although at higher temperatures of 55 °C (Geng et al., 2010). *G. sulfurreducens* was also predominant in the microbial community, indicating that this microorganism likely had an important role in producing the high power densities observed here. *G. sulfurreducens* is well known to be capable of current generation from both acetate and hydrogen gas (Call et al., 2009).

These results demonstrated that power can be produced from particulate substrates such as cellulose, and emphasize the need to carefully develop a microbial community capable of both hydrolysis of particles and power generation. While there are bacteria that have been identified that can both degrade cellulose and produce power (Rezaei et al., 2009), it is more likely that mixtures of bacteria are needed for increasing power production from complex substrates (Ren et al., 2007). Here we found that with proper acclimation that power production with cellulose was only 15% less than that generated using acetate in the same system (Cheng et al., 2010), but it was larger than that produced with complex sources of both soluble and particulate biomass such as wastewaters in these types of MFCs (Pant et al., 2009).

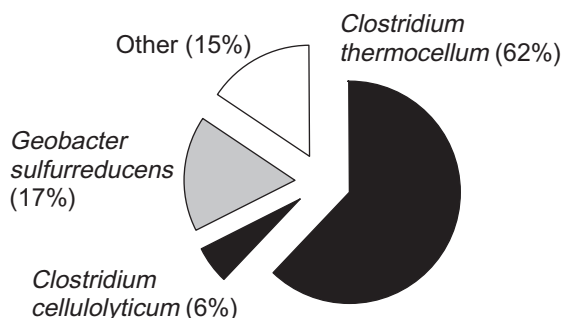


Fig. 3. Bacterial 16S rRNA community analysis of anode biofilm in a cellulose fed microbial fuel cell.

4. Conclusions

The development of a cellulose degrading and current producing inoculum was made possible by first enriching a wastewater inoculum in an MEC. The use of this pre-acclimated inoculum in an MFC made it possible to achieve higher power densities in MFCs than previously obtained using cellulose. A single-chamber MFC produced more power from cellulose than a two-chamber MFC containing an anion exchange membrane and the same electrode spacing. The increased power density was a result of both reduced internal resistance due to a lack of a membrane, and the high mass loading of cellulose in the system. These results show that through careful development of the inoculum and the use of low internal resistance MFCs, that it is possible to generate high power densities from particulate substrates such as cellulose.

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