NOVEL ELECTROCHEMICAL MATERIAL APPLICATIONS AND EXOELECTROGENIC BACTERIA ISOLATION FROM MICROBIAL FUEL CELLS (MFCs)

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by
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A microbial fuel cell (MFC) is a device that uses bacteria as a biocatalyst to directly convert organic matter into electricity. It provides a promising method for producing sustainable bio-energy as well as simultaneously degrading organic waste and wastewater. Corn stover is currently the largest residual biomass source in the US, and it therefore has a great potential as a sustainable and renewable energy source. Two corn stover hydrolysates (acid and neutral) were demonstrated here to produce electricity in single-chambered air-cathode MFCs, with a maximum power density of 371 mW/m² for neutral hydrolysate and 367 mW/m² for acidic hydrolysate. All sugar (monomeric or oligomeric) components were utilized, resulting in ~93% BOD removal efficiency and 20 to 30% of Coulombic efficiency (CE). After applying a diffusion layer onto the cathode surface and increasing the medium conductivity from 10 to 20 mS/cm, the power density could be further improved to 933 mW/m² (neutral hydrolysate) or 971 mW/m² (acidic hydrolysate).

Microorganisms capable of transferring electrons outside the cell to insoluble electron acceptors, such as metal oxides and the anodes of MFCs, are called exoelectrogens. Very few exoelectrogens have been directly isolated from MFCs, and most exoelectrogens known to produce power in an MFC are dissimilatory metal-reducing bacteria (DMRB, e.g. *Shewanella* and *Geobacter*) initially isolated using agar plates containing metals. However, isolation methods based on dissimilatory metal reduction potentially limit the diversity of possible exoelectrogenic bacteria. A special U-tube-shaped MFC was therefore developed to enrich exoelectrogenic bacteria based on
electricity production, with isolation based on dilution-to-extinction methods. A pure
culture was obtained and identified as *Ochrobactrum anthropi* YZ-1 that was unable to
respire using hydrous Fe(III) oxide but produced 89 mW/m² using acetate as the electron
donor in the U-tube MFC, demonstrating a greater diversity of exoelectrogenic bacteria
than simply DMRBs. Strain YZ-1 used a much wider range of substrates as carbon
sources for current production than many DMRBs, including acetate, lactate, propionate,
butyrate, glucose, sucrose, cellobiose, glycerol, and ethanol.

To use MFC-based technologies for real applications in waste and wastewater
treatment processes, one of the greatest challenges is creating a scalable architecture that
provides large surface areas for oxygen reduction at the cathode and bacterial growth on
the anode. A scalable cathode was therefore constructed by coating a tubular
ultrafiltration membrane with conductive graphite paint and a non-precious metal catalyst
(CoTMPP). Using a graphite brush anode with high surface area (A\textsubscript{an,s}=7700 m²/m³) and
two tube cathodes placed inside the reactor (A\textsubscript{cat,s}= 93 m²/m³), the MFC produced 18
W/m³ with a CE = 70 - 74%. Further increases in the surface area of the tube cathodes to
54 cm² (120 m²/m³) increased the total power output (from 0.51 to 0.83 mW), but the
increase in volume resulted in a constant volumetric power density (~18 W/m³).

More cost-effective materials including anion (AEM) and cation (CEM) exchange
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ultrafiltration (UF) cathode. These membranes were made electrically conductive using
graphite paint and a non-precious metal catalyst (CoTMPP) was used. The best
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using an AEM cathode with the conductive coating facing the solution, at a catalyst
loading of 0.5 mg/cm² CoTMPP. The maximum power density was 449 mW/m² (normalized to the projected cathode surface area) or 13.1 W/m³ (total reactor volume), with CE up to 70% in a 50 mM phosphate buffer solution (PBS) using acetate. Using a current collector (a stainless steel mesh) pressed against the inside surface of the AEM cathode and 200 mM PBS, the maximum power produced was further increased to 728 mW/m² (21.2 W/m³). The use of AEM cathodes and brush anodes provides comparable performance to similar systems that use materials costing nearly an order of magnitude more (carbon paper electrodes), and thus represent more useful materials for reducing the costs of MFCs for wastewater treatment applications.
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Chapter 1

Introduction

1.1 Bio-energy Production from Waste and Wastewater

Global economical growth has been built on fossil fuels during the past century, however, this cannot indefinitely continue into the future due to insufficient availability of fossil fuels (1). Moreover, the release of stored carbon by burning of fossil fuels is estimated to increase 3.2 billion tons of carbon dioxide in the atmosphere per year (2). This huge global CO₂ accumulation has clearly caused increases in mean global temperature in the past 50 years. Therefore, to simultaneously solve energy production and CO₂ emission becomes one of the greatest challenges in the 21st century.

Converting biomass and other biodegradable organic matter into energy by green technologies is one of the ideal solutions to achieve renewable energy production and environmental sustainability. Agricultural biomass waste, domestic wastewater, and industrial wastewater contain much energy in the form of biodegradable organic matter. For example, domestic, animal, and food processing wastewaters are estimated to contain a total of 17 GW of power (3). On the other hand, degrading the organic matter in these wastes and wastewaters currently uses a lot of energy. It is estimated that 1.5% of electricity production per year in the US is used for conventional waste and wastewater treatment processes (3). Bio-methane, bio-hydrogen, and bio-electricity production using
different microbial systems and reactors represent three main methods for renewable energy production from waste and wastewater treatment processes.

1.1.1 Bio-methane Production Using Anaerobic Digesters

Anaerobic digestion with methane production is a well-known biological process for waste and wastewater treatment processes using methanogens. Anaerobic treatment systems are normally divided into high-rate systems with a relatively short hydraulic retention time (HRT) and mainly used for many types of wastewaters [e.g. Upflow Anaerobic Sludge Bed (UASB) reactor], and low-rate systems that are normally used to digest wastes and are characterized by a long HRT equal to the sludge retention time [e.g. Continuously Stirred Tank Reactor (CSTR) system] (4). A wide range of wastes and wastewaters have been used for anaerobic digestion with methane production, including municipal solid waste, agricultural wastes, manure, sewage sludge, food and beverage industrial wastewater, and domestic wastewater. The final biogas contains 55 - 75% methane and 25 - 45% carbon dioxide (4). In Europe, biogas production through anaerobic digesters could reach over 15 million m$^3$/d (5). Although anaerobic methane production is a relative mature technology, bioreactor performance will need to be further improved for field applications under conditions such as low temperature, low pH, or more diluted substrates, due to methanogenic microorganisms having a relatively high environmental sensitivity (4).
1.1.2 Bio-hydrogen Production Using Anaerobic Fermenters

The global interest in a hydrogen economy has been built on the promise of using hydrogen as a clean energy carrier to replace hydrocarbon-based fuels for transportation and other end-use applications to decrease the emission of greenhouse gases and other pollutants. A real reduction in CO₂ emissions, however, will require sustainable hydrogen production based on renewable energy sources such as biodegradable organic matter in waste, wastewater, and biomass. The fermentative production of hydrogen from renewable biomass and organic waste presents one strategy of generating renewable hydrogen and assisting in the advancement of a hydrogen-based economy. Acidogenic bacteria, such as some *Clostridium* and *Enterobacter* species (6), are able to ferment certain forms of carbohydrates (e.g. sugars) into hydrogen and other reduced end products (e.g. fatty acids). Hydrogen yields vary within a range from 0.7 up to an optimum stoichiometry 4.0 mol-H₂/mol-glucose, depending on the bacterial community (type of inoculum, such as pure or mixed culture), organic loading, temperature, and pH (6). However, the fermentative hydrogen production process has a relatively low energy recovery (~15 %; 7) due to an incomplete oxidation process. Thus a secondary process (e.g. methane production) is normally needed to completely degrade fermentation end products consisting primarily of acetic and butyric acids.

1.1.3 Bio-electricity Production Using Microbial Fuel Cells (MFCs)

It has been known for almost 100 years that bacteria can be used to generate electricity (8), but most studies on microbial fuel cells (MFCs) didn’t appear until late in
the 20 century (9). In an MFC system, bacteria oxidize reduced substrates, producing electrons that travel through the respiratory chain in the cell membrane and are finally released outside the cell to the anode electrode. The released electrons are then accepted by a terminal electron acceptor (e.g. oxygen or ferricyanide) at the cathode, and thus produce electricity in a closed circuit.

Direct electron transfer outside of the cell between the respiratory chain in the cell membrane and the electrodes was once considered to be inefficient due to non-conductive cell surface structures. Artificial electrochemical mediators were considered essential for achieving a higher electron transfer rate (i.e. current output) and electron recovery (i.e. Coulombic efficiency [CE]) between microbial cells and electrodes. Examples of mediators include 2-hydroxy-1, 4-naphtoquinone, neutral red, and thionin (9, 10). However, MFCs requiring additional mediators, many of which are toxic, would be hard to commercialize.

The first mediator-less microbial fuel cell was developed by Kim et al. in 1999 (11, 12), by using a Fe(III)-reducing bacterium, *Shewanella putrefaciens*. From then on, more and more studies have been focusing on this type of system, since mediator-less MFCs provide a more practical and promising approach to recover electricity from organic waste and wastewater through microbial systems. In a mediator-less MFC, electrons are directly transferred from the cytoplasmic membrane inside the bacteria cell to the extracellular insoluble electrode without any additional mediators. Those electricity-generating bacteria are termed exoelectrogens, or exoelectrogenic bacteria, due to their ability to directly transfer electrons exocellularly. Except as noted, a reactor using
Diverse functional groups of bacteria, including iron-reducing bacteria, denitrifiers, and sulfate reducers, have been found to have exoelectrogenic activities in MFCs (13-17). Many different types of waste organic matter have been used as the energy source for power production, including various carbohydrates, fatty acids, alcohols, and proteins (18-22). At the same time, much effort has been put into improving the system performance, optimizing scalable architecture, and developing cost-effective materials for making an MFC competitive for future renewable energy generation. However, as an emerging technology, there are still many challenges for real MFC applications. Four of the most important areas for current development of MFC technologies are: organic source exploration, the study of exoelectrogenic bacteria and microbial communities, developing economical materials, and reactor scale-up.

1.2 Summaries on MFC Research

My PhD thesis focused on exploring new biodegradable waste materials as MFC energy sources, developing new methods to isolate and identify exoelectrogenic bacteria, developing novel electrode materials with an economic cost, and optimizing a scalable architecture for future MFC applications.
1.2.1 Electricity Production from Steam-Exploded Corn Stover

Although a wide range of waste materials can be converted into energy in MFCs, in order to make MFCs economically viable compared to energy produced by oil and other methods, a suitable energy source should be inexpensive, abundant, sustainable, and collectable. One promising source of agricultural waste biomass is corn stover (the stalk, husk and leaves remaining after the harvesting of corn). With an estimated annual production of 250 million dry tons of biomass, corn stover is currently the largest waste biomass resource in the US, with more than 90% currently left unused in the fields (21). Electricity was generated from the hydrolysates (liquefied biomass produced by steam explosion) of the cellulose and hemicellulose portion (70% of raw corn stover) in MFCs. All sugars (monomeric or oligomeric) were completely utilized, with overall biochemical oxygen demand (BOD) removal efficiencies greater than 90% (21). The highest power produced in an air-cathode single-chambered MFC from corn stover hydrolysates was 971 mW/m² with 20 - 30% of organic matter contained in the hydrolysates converted into energy (21). With this lab achievement and an assumption of 150 million dry tons of corn stover available annually, 4.6×10¹⁰ kWh/year could be produced, equal to 52 power plants generating 100 MW each, worth $6.9 billion per year (assumes $0.15/kWh) (21). Therefore, using corn stover to produce electricity in MFCs provides a new approach for converting biomass to renewable energy other than producing ethanol and biodiesel. (The research was published in Energy and Fuels, and is described in Chapter 3.)
1.2.2 An Exoelectrogenic Bacterium Isolated Using a U-tube MFC

To further advance our understanding of MFCs as well as increase the application potential of MFC technologies, we need to investigate the real diversity of exoelectrogens functioning in MFCs and to obtain those species with high electricity generation abilities. However, few exoelectrogens have been directly isolated from MFCs, and most of these were obtained by plating using Fe(III) as an electron acceptor. This has intrinsically limited the diversity of bacteria capable of exoelectrogenic activities in MFCs since the mechanisms for electron transfer to Fe(III) oxides and the anodes of MFCs now appear to not be exactly the same. Therefore, I developed a novel U-tube MFC system and used it to isolate exoelectrogenic bacteria by directly testing electricity generation activities through dilution-to-extinction methods. This provided the first such isolation device and a new method specifically for obtaining exoelectrogenic species capable of electricity generation. A pure isolate was obtained and identified as *Ochrobactrum anthropi* YZ-1 by its 16S rDNA sequencing and phenotypic characterization results (17). It was further shown that this bacterium is not capable of Fe(III) oxide reduction, demonstrating there is a greater diversity of exoelectrogens than previously suspected. The highest power density achieved by isolate YZ-1 in the U-tube reactor was 89 mW/m$^2$ using acetate as the electron donor, which is comparable to that produced by other pure exoelectrogenic culture so far reported in this system (17). However, YZ-1 showed a much wider range of substrates able to be used in MFCs than other microbes that reduce iron. For example, it uses acetate, propionate, butyrate, lactate, glucose, sucrose, cellobiose, glycerol, and ethanol (17). Such substrate diversity indicates its potential usefulness for degrading
various waste materials and complex waste mixtures. This new U-tube MFC system will provide a useful method for obtaining additional exoelectrogenic microorganisms not obtainable by conventional plating techniques. (The research was published in *Applied and Environmental Microbiology*, and is described in Chapter 4.)

### 1.2.3 Tubular Membrane Cathodes for Scalable Power Generation in MFC

For eventually applying MFCs for real waste and wastewater treatment, one of the greatest challenges is creating a scalable architecture that provides large surface areas for oxygen reduction at the cathode and bacterial growth on the anode in an economical way. Another limitation of existing air-cathode MFC systems is the catalyst. Platinum (Pt) is widely used in current laboratory systems to catalyze oxygen reduction on the air-cathode, but it is not suitable for large scale systems due to its high cost. To solve the cathode scale up problem, I developed a tubular ultrafiltration membrane that could conduct electricity and catalyze oxygen reduction via a graphite coating and a non-precious metal catalyst (CoTMPP). This tubular cathode configuration provided ~100 m²/m³ of specific surface area in an air-cathode MFC, which was equal to 4 times the specific surface area provided by the traditional carbon paper cathode in the same reactor volume, but it cost 74% less (per m²) (23). When a high surface area graphite brush anode was used (7700 m²/m³) with two tube cathodes placed inside the reactor (93 m²/m³), the MFC produced 17.7 W/m³ with a high electron recovery efficiency of 70 - 74%, which is among the highest efficiencies achieved by air-cathode MFC systems (23). Further increases in the surface area of the tube-cathodes to 54 cm² (120 m²/m³)
increased the total power output (from 0.51 to 0.83 mW) (23). These results demonstrated that an MFC design using tubular cathodes coated with non-precious metal catalysts and brush anodes, is a promising architecture that is intrinsically scalable for creating larger systems. (The research was published in *Environ. Sci. Technol.*, and is described in Chapter 5.)

### 1.2.4 Ion Exchange Membrane Cathodes for Scalable MFC

Although tubular cathode architecture is promising for the future scale-up of MFCs, the performance of tubular ultrafiltration membrane cathodes has been limited by the high internal resistance, and the costs still need to be further reduced. I therefore tested the performance of two membrane cathodes constructed from two low-cost, low-resistance anion (AEM) and cation (CEM) exchange membranes using graphite paint and a CoTMPP catalyst. The best performance in single-chamber MFCs using graphite fiber brush anodes was achieved using an AEM cathode with the conductive coating facing the solution, at a catalyst loading of 0.5 mg/cm² CoTMPP (24). The maximum power density was 449 mW/m² (normalized to the projected cathode surface area) or 13.1 W/m³ (total reactor volume), with a Coulombic efficiency up to 70% in a 50 mM phosphate buffer solution (PBS) (24). Using a current collector (a stainless steel mesh) pressed against the inside surface of the AEM cathode, and 200 mM PBS, the internal resistance was further reduced and the maximum power produced was increased to 728 mW/m² (21.2 W/m³) (24). The use of AEM cathodes and brush anodes provides comparable performance to similar systems that use materials costing nearly an order of magnitude more (carbon
electrodes plus Pt as the catalyst), and thus represent more useful materials for keeping relatively high power generation as well as reducing the costs of MFCs for wastewater treatment applications. (The research was submitted to *Environ. Sci. Technol.*, and is described in Chapter 6.)
1.3 Literature Cited


Microbial fuel cells (MFCs) without any artificial mediators represent a new method of renewable bio-energy production as well as organic matter degradation for waste and wastewater treatment. In an MFC (Figure 2.1), bacteria grow on the anode, oxidizing reduced substrates and releasing electrons to the anode and protons into the solution. The cathode can form a separate cathode chamber containing a catholyte to react with the electrons by placing a membrane between the anode and cathode (shown by the dashed line in Figure 2.1) as well as be directly exposed to the air to provide oxygen as the terminal acceptor. The anode and cathode need to be connected with a wire and a load for completing the circuit and producing power.
2.1 Renewable Energy Generation using MFCs

The most promising application for MFCs is to convert organic matter contained in waste and wastewater into electricity, while simultaneously degrading the organic concentration to an acceptable level for discharge. Many studies have demonstrated that MFCs can be used for a variety of substrates, including pure chemicals (e.g. fatty acids, alcohols, sugars, proteins, and cellulose [1-4]), complex wastes and wastewaters such as agricultural waste/wastewater (5, 6), domestic wastewater (7, 8), and other types of wastes and wastewaters (9, 10).
2.1.1 Power Output from MFCs

The power output from MFCs varies widely and is a function of the biodegradability of the organic substrate and the reactor architecture. Simple pure carbohydrates normally generate more power than complex substrates or actual wastewaters. By using a cubic single-chambered air-cathode MFC reactor without a membrane, the maximum power densities were 494 mW/m^2 from glucose (11), 488 mW/m^2 using ethanol (12), and 506 mW/m^2 with acetate (13). Proteins, propionate, and butyrate generated lower power compared to glucose, ethanol, and acetate, with the maximum power densities of 269 mW/m^2 using peptone (2), 354 mW/m^2 from bovine serum albumin (BSA) (2), 68 mW/m^2 for propionate (9), and 305 mW/m^2 for butyrate (13). However, using the same type of reactor and same operation conditions, much less power was generated from actual wastewaters, ranging from 80 to 261 mW/m^2 for meat packing wastewater (80 mW/m^2, 2), domestic wastewater (146 mW/m^2, 11), beer brewery wastewater (205 mW/m^2, 9), and animal wastewater (261 mW/m^2, 5). Suitable wastes or wastewaters that can support comparable power production as simple pure chemicals need to be explored for economical MFC applications.

Power and voltage output from MFCs normally increase with the substrate concentration and follow saturation kinetics with different half-saturation constants ($K_s$). For example, the half-saturation constant was 103 mg/L for glucose (11), 141 mg/L for acetate (13), and 93 mg/L for butyrate (13) when using a cubic single-chambered air-cathode reactor without a membrane. Lower power densities were often reported by using actual wastes and wastewaters compared to pure chemicals, since the low concentration
of organic matter in these wastes and wastewaters were far below the saturation concentration and therefore prohibited the power production. Power could be further enhanced with increased organic concentrations in these wastes and wastewaters. MFCs can generate electricity based on sea sediments, but the first reported sediment MFCs only produced $10 - 20 \text{ mW/m}^2$ (14, 15) due in part to a low organic content (2 - 6%, 15). By adding a particulate substrate, chitin, to a sediment type MFC reactor to increase the organic concentration, the maximum power density increased to $74 - 86 \text{ mW/m}^2$, 40 times higher than that produced from the sediment MFC without enhanced substrates (2 mW/m$^2$; 16). The maximum power density from MFCs using beer brewery wastewater also showed an almost linear increase from 29 to 205 mW/m$^2$ when organic concentration was increased from 84 to 2240 mg COD/L (9).

2.1.2 Organic Matter Degradation in MFCs

Compared to bio-hydrogen production through fermentation, MFCs can achieve much higher organic matter degradation efficiencies during the electricity production process (17). In a two-chambered MFC system, more than 95% of organic content was removed from cereal wastewater (10). Using single-chambered air-cathode MFCs, animal and beer brewery wastewater also achieved similarly high COD removal efficiencies up to 92% (5) and 98% (9), respectively. When domestic wastewater was used in the same system, a COD removal efficiency of up to 80% was achieved, indicating the relatively less biodegradability of domestic wastewater than animal and beer brewery wastewater (7).
2.2 Electricity Producing Bacteria in MFCs

The electricity producing bacteria actively functioning in MFCs are characterized with their ability to directly transfer electrons outside of the cell to the anode of the MFCs. Bacteria that are able to make exocellular electron transfer to insoluble materials, chemicals, and metals, are termed as exoelectrogens (18). Different genetic groups of bacteria have been shown to produce electricity in MFCs, including $\alpha$-Proteobacteria ($\textit{Rhodopseudomonas}$) (1), $\beta$-Proteobacteria ($\textit{Rhodoferax}$) (3), $\gamma$-Proteobacteria ($\textit{Shewanella}$ and $\textit{Pseudomonas}$) (19-21), $\delta$-Proteobacteria ($\textit{Aeromonas}$, $\textit{Geobacter}$, $\textit{Geopsychrobacter}$, $\textit{Desulfuromonas}$, and $\textit{Desulfobulbus}$) (22-26), Firmicutes ($\textit{Clostridium}$) (27), and Acidobacteria ($\textit{Geothrix}$) (28).

The mechanisms of the extracellular electron transfer in MFCs needs to be further explored, and so far they can be classified into three categories. One is by direct contact between the cell and insoluble electron acceptor via membrane-bound electron transfer components located in cell outer membranes, such as cytochromes. These electron carriers can receive electrons from proteins in cytoplasm and/or periplasm membranes and transfer them to the insoluble electrode surface. The second is self-excreted soluble electron-shuffling compounds that can transfer electrons from inside of the cell to the outside insoluble electron acceptors. Third, nanowires (conductive pili) can also deliver electrons between the electron carriers located in the cell membrane to the insoluble materials.
2.2.1 Electron Transfer via Membrane-bound Electron Carriers

A gram-negative bacterium has a five-layer cell structure composed of: 1) inside; 2) cytoplasmic membrane (CM); 3) periplasm; 4) outer membrane (OM); 5) outside. To transfer electrons from the respiratory chain to the external electrode, gram-negative bacteria must establish electron transport links across the outer membrane between cells and extracellular insoluble electrodes. One possible way is the expression of electron transport components in the outer membrane, where they can contact directly with the solid electrode surface.

*Shewanella putrefaciens* is an exoelectrogenic bacterium capable of both electricity production and metal oxide reduction. This bacterium has approximately 80% of membrane-bound cytoplasm cytochromes in its outer membrane under anaerobic conditions (29). These electron transport components in the outer membrane are linked to CM cytochromes via periplasm. These OM cytochrome components were composed of four distinct types, and only an 83 kDa *c*-type cytochrome was highly associated with the extracellular electron transfer activity of *S. putrefaciens* (20). Similarly, a 9.6 kDa *c*-type cytochrome was also found in the outer membrane of *Geobacter sulfurreducens*, another well known exoelectrogenic bacterium, under anaerobic conditions, serving as an intermediary electron carrier from the cytoplasmic respiration chain to external insoluble electron accepters such as Fe(III) oxide and MFC electrodes (30).
2.2.2 Electron Transfer via Nanowires

Some exoelectrogenic bacteria, such as *Pelobacter carbinolicus*, lack c-type cytochromes in the outer membrane, but can also transfer electrons to insoluble Fe (III) oxides (31). Conductive pili were therefore proposed to play a more important role than c-type cytochromes in establishing direct contact between cells and electrodes (32). Conducting-probe atomic force microscopy (AFM) revealed that the pili of *G. sulfurreducens* were highly conductive. Furthermore, *G. sulfurreducens* could not produce pili and could no longer reduce external insoluble electron acceptors such as Fe(III) oxide without the gene encoding for a pilin subunit. The possible function of pili was thus considered as biological nanowires, transferring electrons from cells to insoluble materials such as Fe(III) oxides and electrodes in MFC systems (32).

Gorby et al. (33) demonstrated that *Shewanella oneidensis* MR-1 also have electrically conductive pilus-like appendages by using conductive scanning tunneling microscopy (STM). C-type cytochromes (MtrC and OmcA) were shown to play a key role in the conductivity of these appendages, since the appendages produced from mutants lacking genes for c-type decaheme cytochromes MtrC and OmcA were not conductive. Consequently, these mutants were not able to reduce Fe(III) oxide or generate current in MFCs (33).

The conductive nanowires have a significant effect on the mechanism of microbial electricity production in microbial fuel cells. The pili are anchored in the periplasm space and/or the outer membrane of gram-negative cells, thus it is possible for these pili to accept electrons from either periplasmic or outer membrane electron carrying
proteins (32). These nanowires extend the electron transfer chains well beyond the outer surface of the cell for exoelectrogenic bacteria, providing possibilities for these bacteria to make extracellular electron transfer under unfavorable conditions, e.g., living in the outer surface of the anode biofilm where direct contact could not be made between the outer membrane and the electrode surface (33).

2.2.3 Electron Transfer via Self-excreted Mediators

Some exoelectrogenic species can employ an indirect electron transport system by excreting small soluble molecules that serve as electron shuttles. These electron shuttles are capable of undergoing redox cycling, indicating that they can not only receive electrons from the cytoplasm respiratory chain and get reduced, but also can transfer electrons to the external electron acceptor and become reoxidized.

*Pseudomonas aeruginosa* can produce pyocyanin, a phenazine blue pigment as an extracellular electron shuttle (34). Pyocyanin has been shown to be able to serve as an electron shuttle for *P. aeruginosa* with the presence of the anode as an insoluble electron acceptor (35). However, mutant strains lacking the synthesis of pyocyanin were unable to achieve substantial electron transfer and only reached 5% of the power production compared to the wild type (35). More interestingly, these soluble electron shuttles excreted by *P. aeruginosa* could be taken up and used for electron transfer by other organisms. When pyocyanin was added to an MFC system, the power output and growth of two bacteria strains, *Lactobacillus amylovorus* and *Enterococcus faecium*, were stimulated, although these two bacteria couldn’t produce pyocyanin by themselves (35).
This result indicated the possibility of some non-exoelectrogenic bacteria involved in extracellular electron transfer processes in MFCs.

*S. oneidensis* has been recently reported to be able to excrete a redox-active molecule within biofilms, riboflavin, as an electron shuttle transferring electrons from the respiratory chain to external insoluble electron acceptors and alleviating the requirement of direct contact. Removal of riboflavin from biofilms was demonstrated to reduce more than 70% of the electrons transferred to the poised electrode (36).

### 2.2.4 MFC Studies Using Pure Exoelectrogens

Few exoelectrogens have been directly isolated from MFCs, and most of the pure exoelectrogens used in current MFC studies are dissimilatory metal reducing bacteria (DMRBs) that were initially isolated by using solid Fe(III) or Mn(IV) oxides as insoluble electron acceptors (18, 37, 38). Therefore, the extracellular electron transfer activity for metal reduction in the natural environment and electricity generation in MFCs was originally assumed to be identical (26, 38). However, mutants of *S. oneidensis* MR-1 were recently shown to be able to produce electricity but lost the capability to reduce Fe(III) oxide (39). *P. carbinolicus* was similarly found to be capable of Fe(III) reduction, but was unable to produce current in an MFC (40). These results suggest additional bacteria other than DMRBs might be capable of electricity production in MFCs with different mechanisms for extracellular electron transfer. Pham et al. (26) have used various electron acceptors, including Fe(III), for isolating exoelectrogens from MFCs. However, the isolates, including DMRBs, were less than 0.1% of the number of microbes
estimated to be present by molecular analysis, indicating a greater diversity of electricity producing bacteria than only DMRBs.

The power densities generated from pure culture of exoelectrogens vary according to the biochemical characteristics of the bacterium, the substrate type, and the reactor architecture. However, these power densities are usually equal to or much lower than those obtained using a mixed culture under the same MFC conditions of architecture, circuit load (e.g., high internal resistance), or solution (18). In two-chamber MFC reactors with extremely high internal resistance (1286 Ω), *Geobacter metallireducens* produced a maximum power density of 36 - 40 mW/m², similar to that from a wastewater inoculum (38 ± 1 mW/m²) (41). However, the power output was limited by the MFC architecture due to its high internal resistance, not the bacteria. The power densities show differences between mixed cultures and pure strains when the internal resistance of the MFCs is decreased. *S. putrefaciens* was reported to produce a maximum power of 10.2 mW/m² by using a single-chambered MFC with a Mn⁴⁺ graphite anode and an estimated lower internal resistance of 30 - 100 Ω (42). This power density was only 1.3% of that obtained using a sewage sludge inoculum in the same reactor (788 mW/m², 43). When using a two-chambered MFC with ferricyanide as a catholyte, the internal resistance was 3 Ω and the power difference was even larger when comparing an anaerobic sludge inoculum (4310 mW/m²) to a pure culture of *P. aeruginosa* strain KRA3 (28 mW/m²) (21). The power density generated by the pure *P. aeruginosa* was less than 1% of that produced with the mixed culture (21). However, a pure strain will normally achieve a higher Coulombic efficiency (CE) than a mixed culture. For example, *Desulfituromonas acetoxidans* and *G. metallireducens* achieved more than 80% of CE with 82 - 84% for *D.*
acetoxidans and 84% for *G. metallireducens* (22), while *G. sulfurreducens* could recover as much as 95% of the electrons as electricity with acetate as the substrate (23).

Most DMRBs utilize a limited range of substrates for electricity generation. *S. putrefaciens*, for example, incompletely oxidizes lactate to acetate, but cannot further oxidize acetate in MFCs (44). *G. sulfurreducens* (23, 45) completely oxidizes acetate for power generation, but cannot utilize simple sugars. *Rhodoferax ferrireducens* oxidizes glucose and sucrose in MFCs, but cannot use ethanol, glycerol, or butyrate (3, 46). Exoelectrogens with versatile substrates utilization profiles have more promising application potentials as actual wastes and wastewaters normally contain diverse organic substrates.

### 2.3 Improving MFC Performance

For making MFCs as a competitive method for renewable energy production, MFC based technologies must produce high power output while simultaneously achieving good electron recover efficiencies. Power loss can occur in different ways in MFCs (18): activation loss (for initiating the reactions on both electrodes and extracellular electron transfer to the anode), bacteria metabolism loss (due to bacteria acquiring energy by oxidizing the substrate), mass transfer loss (due to limited flux of the reactants to the electrode), and omic losses (due to proton diffusion resistance and charge transfer resistance). Consequently, there are different methods to improve MFC performance and reduce power losses, such as enhancing electron transfer between bacteria cells and the anode, developing better solid-gas-water reaction interfaces on the
cathode, and decreasing the MFC internal resistance for proton diffusion and charge transfer.

### 2.3.1 Improving Anode Performance

The anode potential in an MFC reactor is determined by the enzyme potential of electricity generating bacteria as well as by the interaction among microbial communities. This potential is always less than the standard oxidation potential of the substrate (18). Metal coatings and other non-metallic material treatment methods can improve anode performance. Precipitating Fe(III) oxides onto carbon anodes reduced the acclimation time for power generation in MFCs inoculated with mixed cultures (47). Adding Mn$^{4+}$, Fe$_3$O$_4$, or Fe$_5$O$_4$ and Ni$^{2+}$ to graphite anodes (42, 43, 48) also enhanced power production. One of the most successful modification methods was using a high temperature (700 ºC) ammonia-gas treatment of anodes (49). The ammonia gas treatment increased the positive charge of the anode surface, and most likely increased the adhesion of negative-charged bacteria. When using an ammonia treated carbon cloth inoculated with wastewater, the acclimation time was reduced by 50% and the maximum power density was increased from 1640 (without ammonia treatment) to 1970 mW/m$^2$ (with the ammonia treatment) (49).
2.3.2 Improving Cathode Performance

In an MFC system, the characteristics of the cathode substantially affect MFC performance (50-52). When using oxygen as the electron accepter at the MFC cathode, the theoretical potential is 0.805 V at pH = 7. However, the actual cathode potential for an air-cathode MFC is much less, typically with an open circuit potential (OCP) of ~0.4 V under normal MFC operation conditions (18). Ferricyanide was often used in MFCs to achieve higher cathode potentials. The standard potential for ferricyanide is 0.361 V, theoretically lower than that for oxygen, but ferricyanide can normally produce greater power than oxygen due to its higher reaction kinetics. The highest power density so far achieved (4310 mW/m², based on the anode surface area) was produced by using ferricyanide as the catholyte in a two-chambered MFC system and inoculated by anaerobic granular sludge (21). Other catholytes used in MFCs including ferric iron (53), manganese (54), and permanganate (55). However, oxygen is considered as the most suitable electron acceptor for practical MFC systems because it is free and its use is sustainable (56).

Oxygen reduction on MFC cathode needs a three-phase reaction interface among solid (catalyst), liquid (electrolyte) and gas (O₂). Since O₂ transport through liquid-phase is much more restricted than the gas-phase, exposing one side of the cathode to air (air cathode) achieves significantly higher performance than dissolving air in the cathode electrolyte (aqueous cathode) (57). In a single-chambered air-cathode MFC without a membrane, the maximum power density was 494 mW/m² (CE = 9 - 12%) using glucose (11). Hot-pressing a Nafion membrane onto the cathode decreased the power density to
262 mW/m² (11), while placing a Nafton membrane in solution between the anode and cathode achieved a similar power density (514 mW/m²; 58) to that without a membrane (494 mW/m²; 11). This comparison indicated that the power reduction was not due to the membrane but rather the changed cathode surface characteristics by bonding the membrane to the cathode. With membranes either on the cathode surface or between the electrodes, higher CEs were achieved (40 - 55%) due to less oxygen diffusion from the cathode to the anode (11, 58). Adding a diffusion layer (DL) composed of a carbon/PTFE base layer and successive PTFE layers to the air-cathode without a membrane can improve the reaction interface among three phases at the cathode surface and significantly increased the maximum power density from 538 (no DL) to 766 mW/m² (4 DLs). The maximum electron recovery efficiency with 4 DLs could reach 32% (51).

The cathode surface area is another key factor that influences the performance of MFCs. In a two-chambered aqueous-cathode MFC system, power increased by 24% when the cathode surface area was increased from 22.5 cm² to 67.5 cm² and decreased by 56% when the cathode surface area was reduced to 5.8 cm² (50).

2.3.3 Decreasing Internal Resistance

Decreasing the distance between the anode and cathode will decrease the internal resistance and can increase power generation. Power generation was increased from 720 to 1210 mW/m² (18 to 61 W/m³) by decreasing the distance between the anode and cathode from 4 cm to 2 cm in a single-chambered air-cathode MFC operated in batch mode (59). Further decreasing the electrode space to less than 2 cm could result in
oxygen diffusion from the cathode reaching the anode under these conditions, and this was found to adversely affect power generation. However, when the same type of reactor with 1-cm spacing between the electrodes was fed with a continuous flow directly through the anode towards the cathode, the maximum power density was further increased to 1540 mW/m$^2$ (51 W/m$^3$; 60). The minimum electrode spacing was achieved by directly placing the cathode against the anode with a cloth separator between the electrodes (61). The cloth separator was effective as reducing oxygen diffusion to the anode, but allowed proton diffusion between the electrodes due to its high porosity. Placing two of these cloth-electrode-assemblies (CEAs) on each side of the same type of cubic reactor with a much reduced volume, the volumetric power density achieved as high as to 627 W/m$^3$ (1120 mW/m$^2$) for fed-batch conditions, and to 1010 W/m$^3$ (1800 mW/m$^2$) under continuous flow operation (61).

Increasing solution ionic strength/ conductivity can also reduce internal resistance and enhance power generation. When solution ionic strength was increased from 100 to 400 mM by adding NaCl (i.e. solution conductivity increased from 10 to 40 mS/cm), power output increased from 720 to 1330 mW/m$^2$ in a single-chambered air-cathode MFC (59). Similarly, increasing the phosphate buffer solution concentration from 50 (7.5 mS/cm) to 200 mM (20 mS/cm) also improved power production (49, 62). However, further increasing solution conductivity over 40 mS/cm had a negative effect on MFC performance (63). The decrease in power output at the much higher solution conductivity was due to an increased working potential of the anode, which resulted in a lower circuit voltage. This indicated that bacterial activity was inhibited by this high salinity.
2.4 Scaling-up MFCs

One of the greatest challenges for using MFCs for wastewater treatment is to identify low-cost and highly effective materials and to create a scalable architecture that provides large surface areas for oxygen reduction at the cathode and bacterial growth on the anode (57, 18). The main three materials of the MFC are the electrode, catalyst, and membrane (if necessary). For both the anode and cathode, a suitable electrode material should be highly conductive, non-corrosive, inexpensive, providing high specific surface area (are per volume ratio), easily available, and scalable (18).

2.4.1 Electrode Material and Architecture

Carbon paper or cloth is commonly used in MFCs as electrode materials. While these materials are highly conductive and effective at producing power, they are expensive and not easily used for scale up. For example, the purchase price for carbon cloth or paper ranges from $1000 to $1200/m². A single-chambered air-cathode MFC containing two flat carbon electrodes can produce 494 - 506 mW/m² (12.4 - 12.7 W/m³; no membrane or separator) with glucose or acetate (11, 13), but it has low projected specific surface areas for both electrodes (7 cm², 25 m²/m³) compared to the normal surface areas used in membrane bioreactors (180 to 6800 m²/m³) (64, 65).

Graphite granules, granular activated carbon (GAC), and graphite fiber brushes can achieve higher specific areas as MFC electrode materials (62, 66-69). Graphite granules, for example, with specific surface areas of 817 to 2720 m²/m³, produced 48 W/m³ with acetate and 38 W/m³ with glucose in a packed-bed reactor (67). A graphite
fiber brush is a promising anode material as it can provide high specific surface area and is more economical than carbon electrodes on a surface-area basis. Graphite fiber brushes can have specific surface areas up to 18,200 m²/m³ at a cost of $0.58/m² (based on the surface area of graphite fibers) (62). Using a brush electrode (18,200 m²/m³) in a cubic air-cathode MFC, the highest power density achieved was 73 W/m³ or 2400 mW/m² (based on the cathode projected surface area) (62). Although these graphite fiber materials are highly effective and less expensive for making anodes, they are not easy to be converted into a cathode due to the difficulty of depositing catalysts on the surface and providing air on the interface. In most studies that used graphite granules and GAC as the cathode materials, a non-sustainable chemical (ferricyanide) was used as a catholyte (66, 67, 69).

For MFC applications in waste and wastewater treatment processes, air-cathodes are more promising than chemical catholytes since air is free and sustainable (57). The air-cathode design is more challengeable than the anode as the reaction interface must be optimized among electrons, protons, oxygen, and the catalyst on the cathode surface. Tubular type of cathodes can provide higher specific area than flat configuration. The first reported tubular cathode was made by hot-pressing carbon cloth containing a Pt catalyst to a Nafion membrane, and then wrapping the material around a porous plastic tube. This design only generated 26 mW/m² (1.6 W/m³) with domestic wastewater as a substrate (7). Other materials have been tried, for example, a cylindrical woven graphite cathode placed on a cation exchange membrane (67) and a cation exchange membrane formed tube packed with graphite granules as the cathode material (69), but both approaches used ferricyanide as a catholyte rather than oxygen. Less expensive,
conductive, and flexible materials should be further explored to make tubular cathodes with high oxygen reduction efficiencies.

2.4.2 Catalyst

Platinum (Pt) is widely used in laboratory systems for air cathodes to catalyze oxygen reduction, but it is also considered to be impractical for large scale systems due to its high cost. In electrochemical tests, two less-expensive metal catalysts, Co-tetra-methyl phenylporphyrin (CoTMPP) and iron phthalocyanine (FePc), have been shown to produce comparable or even higher cathode potentials than that obtained with Pt coated cathodes when the current is above 0.2 mA/cm$^2$ (70). Similarly, Cheng et al. (71) also reported that replacing Pt with CoTMPP improved the cathode performance when the current density was above 0.6 mA/cm$^2$. They further examined the effect of CoTMPP as an air-cathode catalyst in fed-batch MFC tests. By using CoTMPP (0.6 mg/cm$^2$), the maximum power density was 369 mW/m$^2$, only 12% less than that achieved with Pt catalyst with a 0.5 mg/cm$^2$ loading, but 9% higher than that with Pt catalyst with a 0.1 mg/cm$^2$ loading (71). However, without a catalyst, the air-cathode in MFCs only produced 93 mW/m$^2$, indicating that using catalysts on the cathode surface has a significant effect on power production of MFCs (71).

The carbon support has been shown to also have an effect on these non-precious metal catalysts. FePc on Ketjenblack carbon (FePc-KJB) showed higher oxygen reduction activity than that on Vulcan XC carbon (FePc-VC), most likely due to a higher surface area for KJB carbon (72). When using FePc-KJB as the MFC cathode catalyst,
the maximum power density (634 mW/m²) was even higher than that achieved by a Pt cathode with Vulcan XC carbon support (593 mW/m²) (72).

2.4.3 Membranes and Separators

Membranes are often used as a separator between the anode and cathode chambers in two-chambered MFCs with dissolved oxygen or ferricyanide as the electron acceptors. By using membranes, the MFC anode can be maintained under a relatively strict anaerobic environment, and the reactor often achieves higher electron recovery efficiency. However, the use of membranes increases the MFC cost and sometimes reduces the MFC performance due to the increased internal resistance contributed by the membrane. Removing a proton exchange membrane (PEM) bonded to the cathode surface in a single-chambered air-cathode MFC reduced the CE from 44 - 55% to 9 - 12%, although the power increased due to a reduction in the internal resistance (11). Other two-chambered systems using ferricyanide or dissolved oxygen with membranes between the anode and cathode chambers have achieved even higher CEs, e.g. more than 80% (3, 73).

Nafion membrane is used in most two-chambered MFCs. However, a Nafion membrane is very expensive ($1400/m²) and not suitable for large-scale applications. Anion exchange (AEM) (58), cation exchange (CEM) (58, 67, 69), ultrafiltration (58) and bipolar (53) membranes are less expensive and have been used in MFCs to separate electrodes and decrease oxygen diffusion into the anode chamber. Kim et al. (58) showed that placing a CEM, AEM, or Nafion™ membrane between the electrodes did not
appreciably affect the internal resistance (84 - 88 $\Omega$), based on a comparison to the same reactor without a membrane (84 $\Omega$). The power generated using an AEM membrane (610 mW/m$^2$) was slightly larger than that obtained using a CEM (480 mW/m$^2$) or Nafion (514 mW/m$^2$) membrane due to charge transfer by negatively charged phosphate groups (58). CEs for all of these membranes ranged from 35 to 72% (58). Fan et al. (61) also demonstrated that a porous cloth placed between the anode and the cathode increased the CE from 35 to 71% at the same current density of 0.6 mA/cm$^2$. By sandwiching the cloth between the anode and cathode, the internal resistance was significantly reduced, resulting in a power density of 627 W/m$^3$ in fed-batch mode and 1010 W/m$^3$ in continuous-flow mode (61).

2.5 Perspective on MFCs

MFC technologies provide a promising method to achieve sustainable energy production as well as environmental protection. Various organic substrates including wastes and wastewaters have been used in MFCs and produced electricity. While their energy output and efficiencies so far are relatively low, a nearly 100,000 times of increase in power production using air-cathode MFCs has been observed in the past 8 years (18). It has been demonstrated that the MFC performance can be further improved by developing better electrodes, decreasing the internal resistance, and optimizing the reactor architecture and operational parameters. However, many challenges are still remaining to make MFCs commercially applicable in bioenergy production and waste/wastewater treatment. Economical materials including electrode materials,
catalysts, and membranes/separators need to be identified and a scalable MFC architecture with high efficiency also needs to be developed using the cost effective materials. Furthermore, the real diversity of exoelectrogens actively functioning in MFCs needs continuously to be discovered. Likewise, the exocellular electron transfer pathways from exoelectrogenic bacteria to insoluble electrodes should be another additional focus of current MFC researches.
2.6 Literature Cited


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Chapter 3

Electricity Production from Steam-Exploded Corn Stover Biomass

Abstract

Electricity generation using microbial fuel cells (MFCs) was examined from corn stover waste biomass using samples prepared through either neutral or acid steam-exploded hydrolysis processes that convert the hemicellulose to soluble sugars. Maximum power densities in fed-batch tests using an air-cathode MFC were $371 \pm 13 \text{ mW/m}^2$ and $367 \pm 13 \text{ mW/m}^2$ for the neutral and acid hydrolysates (1000 mg-COD/L, 250 $\Omega$). Power output exhibited saturation kinetics with respect to fuel concentration, with predicted maximum power densities of $P_{\text{max}}=475 \text{ mW/m}^2$ and half saturation constants of $K_s=347 \text{ mg/l}$ (neutral), and $P_{\text{max}}=422 \text{ mW/m}^2$ and $K_s=170 \text{ mg/l}$ (acid). Coulombic efficiencies (CEs) were comparable to that found using carbohydrates in this type of MFC, with values ranging from 20 to 30% for both hydrolysates. All sugars (monomeric or oligomeric) were completely utilized, with overall biochemical oxygen demand (BOD) removal efficiencies of $93 \pm 2\%$ (neutral) and $94 \pm 1\%$ (acid). Power output could be increased by using a cathode containing a diffusion layer, resulting in maximum power densities of $810 \pm 3 \text{ mW/m}^2$ (neutral) and $861 \pm 37 \text{ mW/m}^2$ (acid). Power was further increased by increasing solution conductivity to 20 mS/cm, resulting in $933 \text{ mW/m}^2$ (neutral) and $971 \text{ mW/m}^2$ (acid) for the two hydrolysates. Additional increases in solution conductivity lowered the anode potential and did
not increase power. These results demonstrate the potential for a new method of renewable energy production based on conversion of biomass to electricity using MFCs.  

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3.1 Introduction

Corn stover is currently the largest waste biomass resource in the United States, consisting of more than one third of the total solid waste produced, including municipal solid waste (1). An estimated 250 million dry tons of corn stover is produced annually (2). Only a small amount of corn stover is reused as animal feed or bedding, with >90% left unused in fields (1). Corn stover typically contains 70% cellulose and hemicellulose and 15-20% lignin (3). The hemicellulose components can be converted to monomeric and oligomeric sugars by a steam explosion process, forming a sugar-enriched liquid hydrolysate fraction. Ethanol can be recovered from the steam-explored biomass liquid, but <47% of carbohydrates can be converted to ethanol (4). Hydrogen can be produced from the liquefied hemicellulose at an overall utilization efficiency of 87-94% of the glucan and xylan, respectively (5), but most of the chemical oxygen demand (COD) remains as fermentation end products consisting primarily of acetic and butyric acids (6).

Microbial fuel cells (MFCs) represent a new method for energy production and organic matter degradation. Electrochemically active bacteria oxidize organic matter at the anode surface, releasing electrons and protons. Those electrons are transferred from the anode to the cathode through an external circuit, while the protons move to the cathode directly through solution. At the cathode, oxygen or other chemicals such as ferricyanide accept the electrons. MFCs can be used to generate electricity from various carbohydrates, including low-molecular sugars such as glucose (7-9), and complex carbohydrates and carbohydrate-containing wastewaters such as sucrose, starch,
molasses, and wastewater from food (cereal) processing plants (10-13). The energy conversion based on Coulombic efficiency (CE), or the percent of electrons recovered from the organic matter, varies widely and is a function of the wastewater and type of MFC. Using single-chamber MFC containing an air-cathode, the CE was 40-55% when a proton exchange membrane (PEM) was used, but the CE was only 9-12% without the PEM due to oxygen diffusion into the anode chamber (7). In contrast, CEs of 83% and 89% have been obtained using two-chamber aqueous-cathode systems containing a PEM (8, 9). Very low CEs have been found even with two-chamber MFCs containing a PEM, with values of 8.1% for an artificial sucrose solution (10) and 27% for a cereal processing wastewater (13).

In this study, we investigated whether MFCs could be used for electricity production from corn stover hydrolysates. While it seemed reasonable that this sugar-enriched material could be used, the power densities produced with this material, relative to those obtained with other pure compounds and wastes, were not known. Power densities were examined using a single-chamber, air-cathode MFC lacking a PEM that has been previously shown to produce 494 mW/m² (CE = 9-12%) with glucose, and 146 mW/m² (CE = 20%) with domestic wastewater (7). This same system with a PEM produced maximum power densities of only 262 mW/m² (CE = 40-55%) with glucose, and 28 mW/m² (CE = 28%) with domestic wastewater (7). Performance of the MFC with two different corn stover hydrolysates was evaluated in terms of power density, CE, biochemical oxygen demand (BOD)/COD removal, and color removal. To try to increase power production using these substrates, two methods were tested that have been shown
to increase power in this system: increasing the solution conductivity and using a new type of cathode coating (diffusion layer).

3.2 Materials and Methods

3.2.1 Corn Stover Pretreatment via Steam Explosion

Corn stover was soaked in water (neutral pretreatment) or in 1.2% (w/v) sulfuric acid (acidic pretreatment) for ca. 2 h. After draining and pressing out the residual water, approximate corn stove samples (1400 g for neutral, 2500 g for acid) were treated with high-pressure steam in a 4-L steam-explosion reactor (14) at final temperatures and residence times of 220 °C and 3 min (neutral) or 190 °C and 2 min (acid). After cooling, the solids were filtered and washed with water to collect the hemicellulose-derived hydrolysates (3 L for neutral, 4 L for acid). Both hydrolysates were subjected to an overliming process (15), during which Ca(OH)₂ was added to remove the lignin-derived phenolics, followed by adjusting the final pH to near 7.0. The organic matter concentration of the acid hydrolysate (82,200 ± 4400 mg-COD/L) was originally 1.65 times larger than that of the neutral hydrolysate (49,900 ± 2300 mg-COD/L) (Table 3.1). Before being used in tests, the samples were diluted to the same initial COD concentration (250-1000 mg-COD/L, as indicated) using a buffered nutrient medium (pH=7.0; 16) containing the following: NH₄Cl (0.31 g/l), KCl (0.13 g/l), NaH₂PO₄·H₂O (2.93 g/l), Na₂HPO₄ (4.09 g/l); and metal (12.5 ml) and vitamin (12.5 ml) solutions. The
conductivity was adjusted to 10 mS/cm using NaCl as solution conductivity has been shown to affect the maximum power density in this type of MFC (17).

3.2.2 MFC Construction

Two single chamber, membrane-free MFCs were used in this study; they consist of an anode and a cathode placed on opposite sides of a plastic cylindrical chamber (effective volume of 28 ml), as previously described (7). The distance between the two electrodes was 4 cm, with each electrode having a projected surface area of 7.1 cm$^2$. The anode electrode was made of plain Toray carbon paper (without wet proofing; E-Tek, U.S.A.). Unless stated otherwise, the cathode was made of carbon cloth with 0.5 mg/cm$^2$ of Pt on one side (20% of Pt/C catalyst, with water proofing, Vulcan XC-72, De Nora North America, Inc.).

3.2.3 MFC Tests

MFCs were inoculated with domestic wastewater (5 mL, ~300 mg-COD/L) and a nutrient medium (23 ml; 16) containing glucose (1 g/l). Following stable power generation from glucose (after 5 batch cycle operations, 140 h), the substrate was switched to a medium containing a corn stover hydrolysate. The system was considered to be operating under steady state conditions when the maximum voltage output of one batch cycle was reproducible after filling the reactor with fresh medium at least two
times. The medium in the reactor was refilled when the voltage dropped below ~ 20 mV (resistances of 40-500 Ω) or ~ 40 mV (1000-3000 Ω).

A series of experiments were conducted to study the effects of substrate concentration (250, 450, 800 and 1000 mg-COD/L), and circuit resistance (40-3000 Ω) on electricity production. For each initial substrate concentration (250, 450, 800 and 1000 mg-COD/L), different circuit resistances (40-3000 Ω) were used to measure the corresponding voltage output and power density for determination of polarization and power density curves. The optimal resistance was then determined as the resistance at which the power density-current density curve of each substrate concentration achieved the peak value. The performance of the reactor at a high COD concentration was then examined in terms of Coulombic efficiency, organic matter degradation, color removal, and final total suspended solids (TSS) concentration of the effluent.

To increase power output, two different approaches were used. First, the solution conductivity was increased from 10 to 40 mS/cm using NaCl, because increases in the solution conductivity has been shown to increase power density in an MFC using glucose as the substrate (17). An increase in the maximum power density with solution conductivity demonstrates that power generation is limited by the solution chemistry and not by bacterial kinetics. Second, the carbon cloth cathode was coated on the air-facing side with a diffusion layer according to the procedure of Cheng et al. (18). The diffusion layer coating consists of a carbon/ poly(tetrafluoroethylene) (PTFE) base layer containing carbon powder (Vulcan XC-72) and PTFE (30 wt % solution), and four overlying layers of PTFE (60 wt % solution) (18).
3.2.4 Analytical Techniques

All samples were collected and analyzed when voltage output of each batch cycle reduced to less than ~20 mV (40-500 Ω resistors) or ~40 mV (1000-3000 Ω). Chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspend solid (TSS) and color of the samples were measured according to standard methods (19). Samples were centrifuged (Eppendorf Centrifuge 5403; 2750 × g, 20 min) for soluble COD (SCOD) tests, and filtered using 0.2 μm pore-diameter cellulose syringe filters (Corning) to remove bacteria before color measurement.

Samples analyzed for the concentration of sugar and volatile fatty acids (VFAs) were centrifuged (Eppendorf Centrifuge 5403; 2750 × g, 20 min), and the supernatant filtered through 0.2 μm pore-diameter nylon syringe filters (Acrodisc) immediately before analysis. To quantify total sugars, samples were first hydrolyzed in 2.4% (w/v) H₂SO₄ at 121 °C (148 kPa) for 1 h to convert oligomeric sugars into monomeric sugars (20). After neutralization with calcium carbonate solid and filtration through 0.45 μm filters, the hydrolyzed samples were then analyzed using by high-pressure liquid chromatography (HPLC; Agilent 1090 system, Palo Alto, CA) using a refractive index detector and an HPX-87P lead-based column and deashing guard column (Biorad, CA) at 85 °C. Deionized water was used as the mobile phase at a flow rate of 0.6 ml/min. Monomeric sugars were determined similarly without the acid-hydrolysis step. VFAs in the spent medium were analyzed with a HPLC (Agilent 1050 System, Palo Alto, CA) equipped with a UV detector set at 215 nm (Aminex HPX-87H column, Biorad, CA) at
45 °C, at a flow rate of 0.6 ml/min using 4 mM H₂SO₄ mobile phase. The detection limit for VFAs measurement was 0.1 mM.

### 3.2.5 Calculations

Voltage ($V$) was measured across an external resistor using a multimeter with a data acquisition system (2700, Keithly, U.S.A.). Current ($I$) was calculated from $I=V/R$ and power ($P$) according to $P = IV$, where $R$ is the external circuit resistance. Power and current density were normalized by the projected area of the anode (7.1 cm²). Electrode potentials were measured using a multimeter (83 III, Fluke, U.S.A.) and a reference electrode (Ag/AgCl; RE-5B, Bioanalytical systems, U.S.A.). The Coulombic efficiency was calculated by comparing the actual coulombs produced (calculated by integrating the current over time) to the theoretical coulombs production based on the measured total COD removal, assuming a conversion factor of 8 g of COD per mole of electrons, and Faraday’s constant (96485 C per mole of electrons). Energy recovery was calculated by comparing the actual power produced (integrating power output over time) to the theoretical energy contained in the consumed substrates (based on total COD removal), assuming a conversion factor of 1.07 g-COD/g-glucose, and an enthalpy change of -2801 kJ/mol-glucose under standard condition (19).
3.3 Results

3.3.1 Power Produced Using Corn Stover Hydrolysates

A repeatable cycle of power generation was immediately generated after switching from the glucose medium to either the neutral or acid hydrolysate (Figure 3.1). Polarization curves obtained by varying the external circuit resistance (40-3000 Ω) showed that maximum power densities were the same for both hydrolysates at a COD = 1000 mg/L, with 371 ± 13 mW/m² obtained for the neutral hydrolysate (current density of 1446 mA/m², R = 250 Ω) and 367 ± 13 mW/m² for the acid hydrolysate (1439 mA/m², 250 Ω) (Figure 3.2).

The maximum power obtained at different initial COD concentrations fit a Monod type of curve. The predicted maximum power density for the neutral hydrolysate was $P_{\text{max}} = 475$ mW/m², with a half-saturation constant of $K_s = 347$ mg/l ($R^2 = 0.990$). For the acid hydrolysate, the predicted maximum power density was similar ($P_{\text{max}} = 422$ mW/m²) but the half saturation constant ($K_s = 170$ mg/L) ($R^2 = 0.999$) was nearly half that obtained with the neutral hydrolysate (Figure 3.3). A lower half saturation constant $K_s$ showed that the acid hydrolysate could produce more power than the neutral hydrolysate at the same lower substrate concentration.

3.3.2 Organic Matter Degradation

After a complete cycle of power generation, sugars (monomeric or oligomeric) were found to be completely degraded. In addition, there were no detectable volatile
organic acids (acetate, lactic, formic, propionic, and butyric) in solution. Total COD removal efficiencies ranged from 60 to 70%; while soluble COD removals were 68 ± 2% and 76 ± 5%, for the neutral and acid hydrolysates, respectively (Figure 3.4 parts A and B). BOD removal efficiencies were 93 ± 2% (neutral) and 94 ± 1% (acid) (Figure 3.4C).

The physical characteristics of the liquid following a complete cycle of power generation were examined. TSS concentrations of the effluents increased in proportion to initial substrate concentrations, with different production rates for the neutral hydrolysate (0.09 ± 0.01 mg-TSS/mg-COD-removed) and the acid hydrolysate (0.06 ± 0.00 mg-TSS/mg-COD-removed) (Figure 3.5A). The color removal efficiencies for both hydrolysates increased with initial substrate concentration. At the highest substrate concentration of 1000 mg-COD/L, the neutral and acid hydrolysates initially had 430 and 290 chroma units (Hazen). The effluents for the neutral and acid hydrolysates had 340 and 200 chroma units, achieving removal efficiencies of 21 and 30% respectively (Figure 3.5B). At the lowest substrate concentration of 250 mg-COD/L, the influents for both hydrolysates were much lower, with color intensities of 85 (neutral) and 75 (acid) chroma units, and there was no measurable color removal.

3.3.3 Coulombic Efficiency

The overall Coulombic efficiency was a function of either substrate concentration or external circuit resistance. When initial substrate concentration increased from 250 to 1000 mg-COD/L, at the fixed optimal external resistances, the CE decreased slightly from 29.5 to 23.3% for the neutral hydrolysate and from 25.6 to 19.3% for the acid
hydrolysate (Figure 3.6A). At a fixed initial substrate concentration (1000 mg-COD/L), but at different external resistances, the CE varied over a wider range and there was an almost linear increase in the CE with current density. The CE increased from 14.2 to 29.2% with current density from 534 to 2393 mA/m² for the neutral hydrolysate and from 12.3 to 26.9% with current from 560 to 2455 mA/m² for the acid hydrolysate (Figure 3.6B).

### 3.3.4 Improving Power Output

By adding a diffusion layer to the cathode, the maximum power produced for the neutral and acid hydrolysates (1000 mg-COD/L) increased by more than 100%. For the neutral hydrolysate, maximum power density increased from 371 ± 13 mW/m² (1446 mA/m²) to 810 ± 3 mW/m² (2137 mA/m²). The maximum power for the acid hydrolysate increased slightly more, from 367 ± 13 mW/m² (1439 mA/m²) to 861 ± 37 mW/m² (2202 mA/m²) (Figure 3.7).

When solution conductivity was increased from 10 to 20 mS/cm, maximum power densities for both neutral and acid hydrolysates increased to 933 and 971 mW/m², respectively (Figure 3.8A). However, when the solution conductivity was increased to 30 or 40 mS/cm, the maximum power densities decreased. At solution conductivity of 40 mS/cm, maximum power densities decreased to 674 mW/m² for the neutral hydrolysate and to 743 mW/m² for the acid hydrolysate. These values were lower than those obtained at a solution conductivity of 10 mS/cm (Figure 3.8A).
It has previously been shown that the increase of solution conductivity will decrease the internal resistance (17), which decreases the ohmic potential loss and can increase the cell voltage and power output if the system is not limited by bacterial kinetics (i.e. the rate at which the substrate can be degraded). To understand the adverse effect of conductivity on power, the working potentials of the anode and cathode were examined using a multimeter and a reference electrode located in the middle of the fuel cell chamber (acid hydrolysate, 1000 mg-COD/L). When solution conductivity increased from 10 mS/cm (equivalent to 0.6% NaCl) to 20 mS/cm (equivalent to 1.2% NaCl), the anode working potential remained essentially constant but the cathode potential increased, resulting in a greater overall circuit voltage and power density (Figure 3.8B). When solution conductivity was increased to 30 or 40 mS/cm (1.8 and 2.4% NaCl), the anode working potential increased while the cathode working potential remained relatively stable, resulting in a lower overall voltage and power density. This increase in the anode working potential demonstrated that the activity of the electrochemically active bacteria on the anode surface was inhibited by solution conductivities of 30 or 40 mS/cm.

3.4 Discussion

Electricity was generated using both the neutral and acid corn stover hydrolysates with maximum power densities similar to the highest power densities achieved in same MFCs using pure compounds. The predicted maximum power densities achieved here were 475 and 422 mW/m² for the neutral and acid hydrolysates, respectively. The same MFC system produced 494 mW/m² with glucose (7), while power output was lower (349
mW/m²) with butyrate and domestic wastewater (146 mW/m²) (7, 21). Adding a diffusion layer to the cathode increased the maximum power density to 810 ± 3 mW/m² (neutral) or 861 ± 37 mW/m² (acid) (1000 mg-COD/L) respectively. These values are similar to the maximum power density of 800 mW/m² found with glucose using this modified cathode (18). These comparisons demonstrate for the first time that waste materials can produce power densities similar to those obtained with pure compounds. Our findings also indicate that the microbial consortium in the wastewater inoculum can utilize both the oligomeric sugars in the neutral hydrolysate and the monomeric sugars in the acid hydrolysate equally well for power generation. Thus, the acid is not required for the steam-explosion process, simplifying the biomass pretreatment process needed for power generation in a MFC.

While modifying the cathode increased power in a manner similar to that previously described using glucose (18), increasing solution conductivity to 30 mS/cm or more did not increase the maximum power. The greatest power density achieved here was 971 mW/m² for acid hydrolysate at medium conductivity of 20 mS/cm (equivalent to 1.2% NaCl), but this decreased to 789 mW/m² at 30 mS/cm. Liu et al. (17) found that increasing the solution conductivity to 40 mS/cm (using NaCl) increased power output with acetate up to 1330 mW/m² in the same type of MFC. Oh and Logan (22) also found, using a two-chamber MFC, that maximum power was increased with solution conductivities up to 40 mS/cm (using KCl) but power decreased at higher solution conductivities. The decrease in the power output at high solution conductivities shown here was due to the increased working potential of the anode, which resulted in a lower circuit voltage. While the bacterial activity here was reduced by the higher salt
concentration, it may be possible to overcome this limitation in future tests by gradual acclimation of the culture to higher salt concentrations.

3.4.1 Efficiency of Organic Matter Removal

The conversion of organic matter to electricity, on the basis of BOD removal, was relatively high with >93% of the BOD removed for both hydrolysates. This BOD removal efficiency was much higher than that found for domestic wastewater (78 ± 2%, 23), indicating the relatively good biodegradability of the high-sugar content hydrolysates. The lack of complete BOD removal was likely due to more slowly degraded nonsugar components of the hydrolysates or compounds that could not be degraded under anaerobic conditions. While all sugars (oligomeric or monomeric) were completely utilized during in-batch tests, the hydrolysates also contained soluble lignin and small amounts of furfural and hydroxymethyl furfural (HMF). That latter materials contributed to the remaining COD and likely some of the remaining BOD. The overall COD removal efficiencies were only 60% and 70% for neutral and acid hydrolysates, respectively.

3.4.2 Implications for Using Corn Stover as a Source of Renewable Energy

These studies demonstrate that there is great potential for electricity generation using corn stover biomass. Such energy production could be important as a result of the large amount of this material generated, estimated to be 150 million tons per year (3). Assuming all the carbohydrates in the corn stover (70% by mass, 5) can be completely
hydrolyzed to glucose (neglecting the energy input for the corn stover hydrolysis process), and that they are all removed in the MFC process at an energy recovery of 10% (the maximum obtained here), $4.6 \times 10^{10}$ kWh/year could be produced, equal to 52 power plants generating 100 MW each, worth $6.9$ billion per year (assumes $0.15$/kWh, 24). There are obviously many technical challenges to be faced in achieving renewable energy using this waste resource separate from the specific technology examined here, such as reducing the cost for the collection and transportation of corn stover from the field, minimizing the energy expended for the steam explosion hydrolysis, and scaling up the process in an economical manner. In addition, energy recovery in the MFC process needs to be improved to be competitive with other more-mature technologies, such as ethanol production (47% efficiency, 4) and anaerobic digestion in combination with combustion (25-35% efficiency). However, the current study suggests that, even with modest capture efficiencies of the energy in this waste biomass, a substantial amount of electricity could be generated from this material. Further research should be conducted to examine methods to increase the efficiency of BOD removal, color removal, and the Coulombic efficiency using corn stover hydrolysates in MFCs.

3.5 Conclusions

The feasibility of electricity generation from corn stover waste biomass was demonstrated using single-chamber, air-cathode MFCs and diluted corn stover hydrolysates. It was shown that the maximum power densities of $371 \pm 13$ mW/m$^2$ and $367 \pm 13$ mW/m$^2$ were similar for the neutral and acid corn stover hydrolysate (1000 mg-
COD/L, 250 Ω), respectively, using a standard air-cathode. However, power output was increased by using a newly developed cathode coating (diffusion layer) to $810 \pm 3$ mW/m$^2$ for the neutral hydrolysate, and $861 \pm 37$ mW/m$^2$ using the acid hydrolysate. Additional increases in maximum power densities were possible by increasing the solution conductivity to 20 mS/cm, resulting in $933$ mW/m$^2$ (neutral) and $971$ mW/m$^2$ (acid). All sugars (monomeric or oligomeric) were completely utilized, with overall BOD removal efficiencies of $93 \pm 2\%$ (neutral) and $94 \pm 1\%$ (acid). From these lab results it was concluded that corn stover hydrolysates have an excellent potential to be used as substrates for renewable and sustainable electricity generation with MFC technologies.

3.6 Acknowledgements

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3.7 Literature Cited


3.8 List of Figure and Table Captions

Table 3.1: Composition of acid and neutral corn stover hydrolysates.

Figure 3.1: Power generated using neutral or acid hydrolysates at different COD concentrations (250, 450, 800 and 1000 mg-COD/L as indicated).

Figure 3.2: Power density and voltage as a function of current density obtained by varying the external circuit resistance (40 – 3000Ω) at a fixed substrate concentration of 1000 mg-COD/L. (Error bars ±S.D. based on averages measured during highest power output in two separate batch experiments).

Figure 3.3: Maximum power density as a function of initial substrate concentration. (Error bars ±S.D. based on averages obtained during highest power output in three or more separate batch experiments).

Figure 3.4: Organic matter removal efficiency in terms of total and soluble COD, and total BOD as a function of initial substrate concentration. (Error bars are ±S.D. based on samples analyzed in duplicate (TCOD and SCOD) or triplicate (TBOD)).

Figure 3.5: Physical characteristics of effluents in terms of TSS concentration and Chroma removal as a function of initial substrate concentration. [Error bars are ±S.D.
based samples analyzed in duplicate (Chroma removal and influent chroma) or triplicate (TSS).

Figure 3.6: Coulombic efficiencies as a function of (A) initial substrate concentration, and (B) current density.

Figure 3.7: Effect of a cathode diffusion layer (DL) on maximum power densities as a function of current density. (Error bars ±S.D. based on average power output in two separate batch experiments).

Figure 3.8: Effect of conductivity (10 – 40 mS/cm) on (A) maximum power densities and (B) electrode potentials using acid corn stover hydrolysate (vs Ag/AgCl reference electrode; 195 mV vs NHE).
Table 3.1: Composition of acid and neutral corn stover hydrolysates.

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Chapter 4

An Exoelectrogenic Bacterium *Ochrobactrum anthropi* YZ-1 Isolated Using a U-tube Microbial Fuel Cell

Abstract

Exoelectrogenic bacteria have potential for many different biotechnology applications due to their ability to transfer electrons outside the cell to insoluble electron acceptors, such as metal oxides, or the anodes of microbial fuel cells (MFCs). Very few exoelectrogens have been directly isolated from MFCs, and all of these organisms have been obtained by techniques that potentially restrict the diversity of exoelectrogenic bacteria. A special U-tube-shaped MFC was therefore developed to enrich exoelectrogenic bacteria with isolation based on dilution-to-extinction methods. Using this device, we obtained a pure culture identified as *Ochrobactrum anthropi* YZ-1 based on 16S rRNA gene sequencing and physiological and biochemical characterization. Strain YZ-1 was unable to respire using hydrous Fe(III) oxide but produced 89 mW/m² using acetate as the electron donor in the U-tube MFC. Strain YZ-1 produced current using a wide range of substrates including acetate, lactate, propionate, butyrate, glucose, sucrose, cellobiose, glycerol, and ethanol. Like another exoelectrogenic bacterium (*Pseudomonas aeruginosa*), *O. anthropi* is an opportunistic pathogen, suggesting that electrogenesis should be explored as a characteristic that confers advantages to these
types of pathogenic bacteria. Further applications of this new U-tube MFC system will provide a method for obtaining additional exoelectrogenic microorganisms that do not necessarily require metal oxides for cell respiration.²

4.1 Introduction

Electricity generation in a mediatorless microbial fuel cell (MFC) is linked to the ability of certain bacteria, called exoelectrogens (“exo-” for exocellular, and “electrogens” for the ability to transfer electrons to insoluble electron acceptors), to transfer electrons outside of the cell to the anode in an MFC (1). Different genetic groups of bacteria have shown exoelectrogenic activity in MFCs, including β-Proteobacteria (Rhodoferax) (2), γ-Proteobacteria (Shewanella and Pseudomonas) (3-5), δ-Proteobacteria (Aeromonas, Geobacter, Geopsychrobacter, Desulfuromonas, and Desulfobulbus) (6-10), Firmicutes (Clostridium) (11), and Acidobacteria (Geothrix) (12). The mechanisms used for exocellular transport of electrons by these bacteria are still being studied. It has been demonstrated that cell-bound outer membrane cytochromes and conductive pili (nanowires) may play a key role in electron transfer for some Geobacter and Shewanella species (13-16). Alternatively, some exoelectrogens, such as Pseudomonas aeruginosa (5) and Geothrix fermentans (12), excrete mediators to shuttle electrons to surfaces.

Many of the exoelectrogens that produce current in an MFC are dissimilatory metal-reducing bacteria (DMRBs) that were originally isolated based on their ability to reduce insoluble metals, such as Fe(III) or Mn(IV) oxides, in the natural environment (1, 17, 18). The mechanisms for electron transfer to metal oxides were originally assumed to be identical to those for electricity generation (10, 18). However, some new evidence suggests that the mechanisms for electron transfer to metal oxides and to MFC anodes are
not always the same. *Shewanella oneidensis* MR-1 is an exoelectrogen capable of both electricity production and Fe(III) oxide reduction. Two mutants of *S. oneidensis* MR-1 (SO4144 and SO4572) were recently shown to be able to produce electricity but to have lost the ability to reduce Fe(III) oxide (19). *Pelobacter carbinolicus* was similarly found to be capable of Fe(III) reduction but was unable to produce current in an MFC (20). These results suggest that different genes may be involved in electron transfer to metal solids and in electron transfer to graphite electrodes.

Few exoelectrogens have been directly isolated from MFCs, and all of the previous methods used conventional plating techniques. However, the use of agar plates is not a selective method for electricity-producing bacteria. *Clostridium butyricum* and *Aeromonas hydrophila* were the first two microorganisms isolated from MFC anodes by plating with soluble Fe(III) citrate (11) or Fe(III) pyrophosphate (10). By using insoluble Fe(III) oxide as the electron acceptor, *Geopsychrobacter electrodiphilus* was isolated from a marine sediment fuel cell (9). Although these isolates have shown electricity generation in MFCs, Fe(III) plating methods eliminate the growth and isolation of other exoelectrogens that may not be able to respire with iron on the plates. General nutrient agar plates were also used for isolation of exoelectrogen from MFCs under aerobic and anaerobic conditions (5), but this method allowed the non-selective growth of nonexoelectrogenic bacteria, making it difficult to choose which colonies should be used in further studies. Therefore, the current isolation methods used to obtain electricity-producing bacteria by plating methods are indirect and potentially biased, and they may not allow for identification of the true diversity of the exoelectrogens functioning in MFCs.
In order to better understand the characteristics of bacteria capable of exoelectrogenic activity in MFCs, we developed a new method to enrich and isolate these bacteria that is independent of the need for metal oxide reduction. Our new device, called a U-tube MFC, was constructed to allow bacteria in suspension to directly settle on the anode, making it theoretically possible to eventually produce current from the initial growth of a single cell. Through repeated dilution to extinction, we showed that this U-tube MFC can be used to directly isolate exoelectrogens according to their electricity-generating ability and not their ability to reduce iron. This approach allows us to enrich and isolate additional exoelectrogenic strains that might not be obtainable by conventional plating techniques.

4.2 Materials and Methods

4.2.1 U-tube MFC Construction

The U-tube MFC was constructed from a straight tube that formed the anode chamber (10 mL), and a U-shaped tube for the cathode chamber (30 mL). The two chambers were separated by a cation exchange membrane (1.77 cm²; CMI 7000; Membranes International Inc., US) and were joined together by a C-type clamp (Figure 4.1). Both the anode and the cathode tubes were made from anaerobic culture tubes (Bello Glass, US) and sealed with butyl rubber stoppers. Placing the anode on the bottom of the vertically aligned anode chamber tube allowed bacteria to be readily deposited directly on the electrode surface. The U shape of the cathode chamber used hydrostatic
pressure to keep the catholyte solution [100 mM K$_3$Fe(CN)$_6$ in 100 mM phosphate buffer solution (PBS)] pressed against the cathode. Dissolved oxygen was not used as sparging would have resulted in gas collection on the cathode. The absence of oxygen in the cathode chamber was important during the growth of a small number of cells because oxygen could have diffused from the cathode chamber into the anode chamber through the membrane.

The anode was plain carbon cloth (type A, E-Tek, US) pretreated using a high-temperature ammonia gas process (21). A piece of the anode (6-cm-long strip) extended outside the tube in order to make an electrical connection. The cathode was made of 15-cm-long plain graphite fibers (no. 292 carbon fiber tow; Fibre Glast, US) wrapped at one end with a titanium wire, with the fiber bundle positioned close to the CEM (Figure 4.1). The wire was extended through the top of the rubber stopper to complete the electrical connection. The graphite fiber cathode provided a much larger surface area (2260 cm$^2$) than the anode electrode (1.77 cm$^2$) to reduce limitations on power generation by the cathode. The anode was connected to the cathode via a 1000 $\Omega$ resistor, unless otherwise noted.

4.2.2 Isolation

The initial inoculum was obtained from the anode of a single-chamber air-cathode cubic MFC (22) operated for more than 1 year (originally inoculated with primary clarifier overflow of a local wastewater treatment plant) fed with a medium containing acetate (1 g/L), NH$_4$Cl (0.31 g/L), KCl (0.13 g/L), and metal salts (12.5 mL/L) and
vitamins (5 mL/L) in a 50 mM PBS solution as described previously (23). The same medium with acetate (1 g/L) was used in U-tube tests except as noted for tests with different substrates. Standard anaerobic techniques were used throughout isolation procedures where possible. The medium was boiled under 1 atm of N₂ before it was dispensed into anaerobic test tubes (Bellco Glass, US) or the anode chamber of U-tube MFCs under N₂. A ferricyanide solution was sparged with N₂ and then put into the cathode chamber of the U-tube MFCs. All of the tubes and MFCs were sealed with rubber stoppers, crimped with aluminum caps, and sterilized by autoclaving. A piece (1 cm²) of the enriched anode from the single-chamber MFC was transferred to an anaerobic tube containing 10 ml of PBS solution (50 mM) and glass beads. The tube was vortexed, producing a suspended cell concentration of \( \sim3\times10^8 \) cell/mL (measured by acridine orange direct counts using fluorescence microscopy). The cell suspension was then serially diluted in 10-fold steps to an end point dilution of \( 10^{-8} \) in anaerobic tubes. A sample (1 mL) from each tube was then transferred to the anode chamber of a U-tube MFC device containing 9 mL PBS nutrient medium and acetate. A U-tube reactor without any cells (containing only sterile medium) was used as an uninoculated control.

The U-tube MFCs were incubated in a constant temperature room at 30°C. Growth of exoelectrogens was monitored by current production. Electricity-producing cultures were incubated until the current peak was observed. The anode from the U-tube MFC containing the highest dilution that produced electricity was then transferred to an anaerobic tube containing sterile PBS, and the isolation procedure (vortexing and dilution) described above was repeated. Before reuse, the U-tubes were cleaned, reassembled, sterilized, and provided with a completely new carbon cloth anode, a new CEM, and a
graphite fiber cathode. This procedure was repeated until the denaturing gradient gel electrophoresis (DGGE) profile used for community analysis showed a single band.

### 4.2.3 DNA Extraction, PCR, DGGE, and Sequence Analysis

One-half of the cell suspension extracted from each U-tube anode that represented the highest dilution with electricity production was used to track community succession by DGGE. DNA was extracted using a PowerSoil™ DNA isolation kit (MO BIO Laboratories, US) according to the manufacturer’s instructions. A 16S rRNA gene fragment of the extracted DNA was then amplified by polymerase chain reaction (PCR) using a 50-µl (total volume) mixture containing GoTaq® Green Master Mix (Promega, US), 1 µM of each primer, 100 ng of DNA template, and sterile deionized water (24). For DGGE analysis, the primers used for PCR were GC968F (5′-CGCCCGCCGCCCCGCGCCCGCGCCCGCCCCCGCCCCAACGCGAAGAACCTTAC-3′) and 1401R (5′-CGGTGTGTACAACGCGCGCGGACACCAACGCGAAGAACCTTAC-3′) (25, 26). The samples were amplified using an iCycler iQ™ thermocycler (Bio-Rad Laboratories, US) and the following thermal profile: 95°C for 4 min; 20 cycles of 30 s at 95°C, 30 s at 60°C, and 1 min at 72°C with a 0.1°C decrease in the annealing temperature per cycle to 58°C; 15 cycles of 30 s at 95°C, 30 s at 58°C, and 1 min at 72°C; and extension for 10 min at 72°C. DGGE was performed using a DCode universal mutation detection system (Bio-Rad Laboratories, US) with a denaturing gradient ranging from 30 to 60% (100% corresponds to 7 M urea and 40% [v/v] deionized formamide). Electrophoresis was performed for 15 min at 30 V and for 13 h at 75 V at 60°C. The obtained gels were silver-stained (25).
Once the DGGE analysis showed a single band, PCR was performed with primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGATCCAGCC-3') (27) to amplify the nearly complete bacterial 16S rRNA gene for sequencing the putative isolate. The DNA amplification was carried out under the following conditions: 95°C for 5 min; 35 cycles of 95°C for 1 min, 57°C for 30 s, and 72°C for 1.5 min; and finally 72°C for 7 min (24). PCR products were purified by a QIAquick gel extraction kit (Qiagen, US), and were ligated and cloned using the TOPO TA cloning kit (Invitrogen, US) according to the manufacturer’s instructions. Plasmids were isolated from randomly selected clone colonies with the QIAprep Spin Miniprep kit (Qiagen, US), and nine plasmid inserts were then sequenced in both directions using an ABI 3730XL DNA sequencer (Applied Biosystems, US) and found to be identical. The obtained 16S rRNA gene sequence was compared to the most closely related strains in GenBank by using the BLAST program. A neighbor-joining phylogenetic tree was constructed using the Molecular Evolutionary Genetics Analysis package (MEGA version 3) (28) with Kimura’s two-parameter method (29). A bootstrap analysis was based on 1000 resamplings.

**4.2.4 Transmission/Scanning Electron Microscopy**

For transmission electron microscope examination, a 5-µl cell suspension of the isolate was negatively stained using 2% of aqueous uranyl acetate on a Formvar carbon-coated copper grid. The grid was air dried and then examined with a transmission electron microscope (JEM 1200 EXII; JEOL) at an accelerating voltage of 80 kV.
Selected MFC electrodes enriched with an isolate were examined using a scanning electron microscope. The electrode samples were fixed with 1.5% glutaraldehyde for 1 h and then postfixed with 1% osmium tetroxide for 30 min. After each fixation step, the samples were washed in 0.1 M cacodylate buffer three times. The fixed samples were dehydrated with ethanol and dried using a critical point drying process in liquid CO₂ (BAL-TEC CPD030, Bal-Tec, US). Samples were then sputter coated with Au/Pd and examined using a JSM 5400 scanning electron microscope (JEOL) at an accelerating voltage of 20 kV.

4.2.5 Physiological and Biochemical Characterization

Carbon source utilization tests were performed with the isolate either using BIOLOG plates (Biolog Inc., US) under aerobic conditions or using U-tube MFCs under anaerobic conditions. For all of the physiological and biochemical characterizations, the isolate was precultivated and enriched on Difco™ nutrient agar plates (for culturing nonfastidious microorganisms; BD company, US) or in Difco™ nutrient broth (BD company, US) at 30°C under aerobic conditions. Cells were washed three times with 50 mM PBS, and the concentration was adjusted to a (5.0 ± 0.5) × 10⁸ cell/mL (as determined by acridine orange direct counting) before tests. Duplicate BIOLOG GN2 MicroPlates containing 95 separate carbon sources were incubated with 150 µl of isolate cell suspensions in each well for 24 h at 30°C. To test the substrate utilization of the isolate for electricity production under anaerobic conditions, stationary-phase cultures of the isolate were inoculated (10/100, vol/vol) to U-tube MFCs containing propionate,
butyrate, lactate, glucose, sucrose, cellobiose, ethanol, or glycerol (1 g/L for all of substrates) as the sole electron donor in 50 mM PBS nutrient medium (23), and the current production was measured using a 1000 Ω resistor.

The denitrification activity of the isolate was determined in anaerobic tubes (in triplicate) containing 10 mM nitrate and 1 g/L acetate at 30°C. One tube without cells was used as an abiotic control. Nitrate reduction was detected by the nitrite spot test using Griess reagents I and II [sulfanilamide and N-(1-naphthyl)-ethylene-diamine-dihydrochloride], and by measurement of the nitrate concentration at 620 nm with a Spectronic 20 spectrophotometer (Bausch and Lomb, US).

The ability of cells to respire using hydrous ferric oxide (100 mM) (30) was investigated using 1 g/L acetate in anaerobic tubes (in triplicate). One tube without cells was used as an abiotic control. All tubes were incubated at 30 °C for 7 days. The reduction of Fe(III) was monitored using a ferrozine colorimetric method based on the production of HCl-extractable Fe(II) (31). Color changes in the ferrozine solution were measured at a fixed wavelength of 562 nm.

4.2.6 Electricity Production

All U-tube MFCs were considered to be fully acclimated when the maximum voltage produced was repeated for at least three batch cycles. The reactor was refilled with fresh anode nutrient medium and cathode ferricyanide solution when the voltage dropped below ~ 20 mV. The voltage (V) of U-tube MFC reactors was measured across a resistor using a data acquisition system (2700; Keithly, US). Current (I) was calculated
using the equation $I = V/R$, where $R$ is the resistance, and maximum current densities were normalized to the anode projected surface area. To obtain the polarization and power density curves and Coulombic efficiency (CE) as a function of current, the external circuit resistance was varied from 250 to 5000 $\Omega$. One resistor was used for at least two separate full cycles of operation. Power ($P = IV$), power density ($P = IV/A_{an}$, where $A_{an}$ is the anode surface area) and CE (defined as the fraction or percentage of electrons recovered as current in one batch cycle versus the total available electrons from the initial input substrate, [e.g. 8 mol e$^-$ per mol acetate]) were calculated as previously described (32). For comparison of power densities achievable in this system, U-tubes were inoculated with domestic wastewater (primary clarifier effluent) and then operated for multiple batch cycles until power generation was stable using the same substrate (acetate) and PBS nutrient medium.

4.2.7 Nucleotide Sequence Accession Number

The 16S rRNA gene sequence determined in this study has been deposited in the GenBank database under accession number EU275247.

4.3 Results

4.3.1 U-tube Isolation of Exoelectrogens

After the anode microorganisms from the acetate-enriched MFC were serially diluted and transferred to eight U-tube reactors containing acetate, current was produced
with lag times following the sequence of dilution ($10^{-1}$ to $10^{-8}$). The lag phase ranged from 20 h long for the lowest dilution ($10^{-1}$) to 100 hour long for the second-highest dilution ($10^{-7}$) (Figure 4.2). The 10-8 dilution did not produce current after 160 h in this first round of dilutions. The lowest dilution ($10^{-1}$) consistently produced the first current peak, and the highest current density, 481 mA/m2 (based on the anode area), was produced at ~70 h. Within 150 h, all of the electricity-producing U-tubes ($10^{-1}$ to $10^{-7}$ dilutions) produced similarly high peak currents ranging from 412 to 532 mA/m2 (Figure 4.2). Sequential current production was repeated in all eight of the dilution-to-extinction cycles, and no current was produced in the uninoculated control (abiotic) U-tube MFCs throughout the study.

The DGGE profiles obtained over the eight enrichment and dilution cycles showed that the diversity of the microbial consortium significantly decreased after five isolation cycles (Figure 4.3). Although the initial inoculum had relatively great genetic diversity based on the appearance of multiple bands, only a single band (band 1) remained after eight cycles, suggesting that only one bacterium was present in the system. This same band was observed for the other seven isolation cycles, indicating that one microbe accounted for a significant fraction of the community in all cycles.

**4.3.2 Sequence and Phylogenic Analysis**

The nearly complete 16S rRNA gene sequence (1446 nucleotides, GenBank accession number EU275247) of the isolate, designated strain YZ-1, was identical between primers 968 and 1401 (i.e., the target locations of primer 968F and 1401R,
respectively) with the sequence obtained from band 1. Phylogenetic analysis of 16S rRNA gene sequences of strain YZ-1 and closely related organisms in the GenBank database showed that strain YZ-1 belongs to the genus *Ochrobactrum* together with *Ochrobactrum anthropi*, *Ochrobactrum cytisi*, and *Ochrobactrum lupini*, with 100% identity to sequences from *O. anthropi* LMG3331$^\top$ (= ATCC 49188$^\top$) and *O. cytisi* ESC1$^\top$ (Figure 4.4).

### 4.3.3 Physiological and Biochemical Characterization of YZ-1

Strain YZ-1 is a gram-negative rod, 1 to 1.5 μm long and 0.4 to 0.6 μm wide, and it is motile by means of a polar flagellum (Figure 4.5A). Selected anodes enriched with strain YZ-1 were examined, and the results showed that cells formed a biofilm on the surface of carbon cloth fibers. In some places, cells colonized, formed multiple layers of biomass, and completely covered the electrode surface (Figure 4.5B, C and D).

Carbon source versatility and nitrate reduction by strain YZ-1 were evaluated and compared to data reported previously for *O. anthropi* (33), *O. cytisi* (34), and *O. lupini* (35) (Table 4.1). Strain YZ-1 had more phenotypic characteristics of *O. anthropi* than of *O. cytisi* and *O. lupini*. Both strain YZ-1 and *O. anthropi* were able to reduce nitrate and aerobically utilize gluconate, galactose, D-fructose, acetate, propionate, butyrate, lactate, ethanol, glycerol, glucose, cellobiose, and sucrose, but were unable to assimilate citrate (24 h), lactose, melibiose, and N-acetylglucosamine. Strain YZ-1 differed from *O. cytisi* in gluconate, citrate, and N-acetylglucosamine assimilation and differed from *O. lupini* in
nitrate reduction and utilization of citrate, lactose, melibiose, galactose, D-fructose, turanose, N-acetylglucosamine, D-arabitol, glycerol, and cellobiose.

Although strain YZ-1 showed exoelectrogenic activity by producing electricity in U-tube MFCs with acetate as the sole electron donor, it did not reduce iron oxide with the same carbon source. Strain YZ-1 was cultivated for 7 days at 30°C with acetate and poorly crystallized hydrous ferric oxide in anaerobic tubes. Samples were taken on days 1, 2, 3, and 7, but no detectable Fe(II) was found.

### 4.3.4 Electricity Production by Strain YZ-1

Electricity was rapidly generated in all U-tube reactors inoculated with strain YZ-1 within a few hours using acetate (1 g/L) as the electron donor. After there was repeatable current production for at least three cycles, the electricity generation ability of strain YZ-1 was compared to that of a mixed-culture inoculum derived from domestic wastewater under the same conditions. Power density and polarization curves showed that YZ-1 produced less power than the mixed culture; the maximum power density were 89 mW/m² (at 1000 Ω, 708 mA/m²) for strain YZ-1 and 539 mW/m² (at 1000 Ω, 1730 mA/m²) for the mixed culture (Figure 4.6). However, strain YZ-1 showed a much higher electron recovery efficiency than the mixed culture. More than 80% of electrons from acetate were recovered as current using strain YZ-1 at 234 to 1027 mA/m², with a CE of 93% at 1027 mA/m². In contrast, only 39 to 46% of available electrons were recovered using the mixed culture over the current range of 639 to 2603 mA/m² (Figure 4.7). *O. anthropi* type strain ATCC 49188 also generated electricity in the U-tube MFC, but the
power produced (45 mW/m², 1000 Ω, 502 mA/m²) was less than that obtained with strain YZ-1.

Strain YZ-1 used a greater diversity of carbon sources than most DMRBs. In U-tube MFCs, strain YZ-1 generated current (142 to 275 mA/m²) using propionate, butyrate, and lactate, although at densities that were 60 to 80% less than those produced with acetate (Figure 4.8). However, YZ-1 showed higher current densities using sugars (monosaccharides and disaccharides) and alcohols, and the maximum current densities were 481 mA/m² for glucose, 413 mA/m² for sucrose, 425 mA/m² for cellobiose, 414 mA/m² for ethanol, and 357 mA/m² for glycerol (Figure 4.8).

4.4 Discussion

By using a U-tube MFC system, we demonstrated that exoelectrogens can be isolated using dilution-to-extinction methods based directly on their ability for electricity generation. U-tube dual-chamber MFCs can be autoclaved and are well sealed so that a sterile and anaerobic environment can be obtained for exoelectrogen growth. Milliken and May (36) reported a similarly shaped MFC that was used for evaluation of electricity production by Desulfitobacterium hafniense based on use a mediator (anthraquinone-2,6-disulfonate) and oxygen at the cathode. Instead of using two symmetrical curled tubes containing floating electrodes inside the tubes, as in the study of Milliken and May, the U-tube isolation device developed here uses a straight anode tube with a flat anode placed at the bottom. This configuration allows a small number of cells in the most diluted samples to settle onto an anode surface so that they can grow and produce current. The
asymmetric U-shape cathode chamber also provides a high ratio of cathode volume to anode volume and has a graphite fiber cathode with a large surface area to increase cathode efficiency, and a chemical catholyte solution is used to avoid oxygen contamination.

Most exoelectrogens that can produce power in an MFC are DMRBs initially isolated using agar plates containing Fe(III). However, strain YZ-1 obtained in this study produced electricity but was incapable of growth with acetate using hydrous ferric oxide (poorly crystallized Fe(III) oxide) in suspension or Fe(III) pyrophosphate on agar plates. Pham et al. (10) used various electron donor and acceptor combinations, including Fe(III) as the electron acceptor, for solid media for isolating exoelectrogens from MFCs. However, the isolates recovered from colonies accounted for less than 0.1% of the microbes estimated to be present by molecular analysis, indicating the selective limitation of traditional plating methods on isolating electricity-producing microorganisms.

The power densities generated from pure cultures of exoelectrogens are usually equal to or much lower than those obtained using a mixed culture under the same MFC conditions of architecture (type of reactor), circuit load (e.g., high internal resistance), or solution (i.e. conductivity) (1). For example, Min et al. (37) reported similar maximum power densities from a wastewater inoculum (38 ± 1 mW/m²) and Geobacter metallireducens (36 to 40 mW/m²) in two-chamber MFC reactors with acetate as the substrate and dissolved oxygen as the electron acceptor. However, the internal resistance is extremely high for this type of reactor (1286 Ω), and thus power is limited by the architecture and load and not by the ability of the bacteria to produce high current. When a single-chamber air-cathode MFC with a Mn⁴⁺ graphite anode and an estimated lower
internal resistance (30 to 100 Ω) was used, *Shewanella putrefaciens* (38) produced a maximum power of 10.2 mW/m². This was only 1.3% of the power output obtained using a sewage sludge inoculum in the same reactor (788 mW/m²) (39). An anaerobic sludge inoculum produced a maximum power density of 4310 mW/m² in a two-chamber MFC using ferricyanide with low internal resistance (3 Ω). In the same type of system, a pure culture of *P. aeruginosa* strain KRA3 produced only 28 mW/m², which was <1% of the power generated with the mixed culture (5). Strain YZ-1 produced a maximum power density of 89 mW/m² in U-tube MFC reactors (internal resistance, 69 ± 4 Ω), which was 17% of the power produced with the mixed consortium. However, strain YZ-1 had a high CE (up to 93%), similar to that observed with *Geobacter sulfurreducens* (95%) with acetate as the substrate (7).

Most DMRBs utilize a limited range of substrates for growth or electricity generation. *S. putrefaciens*, for example, oxidizes lactate to acetate, but it cannot oxidize acetate under anaerobic conditions (40). *G. sulfurreducens* (7, 41) completely oxidizes acetate for power generation, but it cannot utilize simple sugars. *Rhodoferax ferrireducens* oxidizes glucose and sucrose in MFCs, but it cannot use ethanol, glycerol, or butyrate (2, 42). Stain YZ-1 uses a much wider range of substrates as carbon sources (Table 4.1), including acetate, glucose, and cellobiose. These three substrates generated the high current densities with stain YZ-1: 708 mA/m² (acetate), 481 mA/m² (glucose), and 425 mA/m² (cellobiose). The versatile substrate utilization of stain YZ-1 indicates its potential relevance for use with wastewaters that have highly variable compositions.

Electricity generation is a newly identified characteristic of *O. anthropi*. However, *Ochrobactrum* species have been identified as members of anode microbial communities.
in other MFCs using glucose or acetate as a substrate (5, 43). Like another exoelectrogenic bacterium, *P. aeruginosa*, *O. anthropi* is an opportunistic pathogen belonging to *Proteobacteria*. Both of these organisms are facultative bacteria that are capable of aerobic respiration and anaerobic denitrification (33, 44, 45). Furthermore, both of these bacteria are found in the rhizosphere of diverse plants, indicating an association with plant roots, and have the ability to degrade a wide range of environmental pollutants (46). Both microbes can produce exopolysaccharides, a factor which could be relevant to their colonization on the electrode in MFCs. These common characteristics of *P. aeruginosa* and *O. anthropi* suggest exoelectrogenesis may be a selective property for opportunistic pathogens, a situation which should be further explored.

Although additional study is still needed to discover more details about the exocellular electron transfer mechanism(s) used by *O. anthropi* in MFCs, this is the first time an *Ochrobactrum* species has been demonstrated to have the ability to produce electricity. It is suggested that a greater diversity of exoelectrogens may exist in anode communities, and that isolates from these communities could be obtained using U-tube devices rather than traditional metal oxide plating methods. By using different substrates and using U-tube MFCs with lower internal resistances, these isolation devices may allow us to more fully study the diversity of exoelectrogenic bacteria in MFCs.
4.5 Acknowledgements

This project was funded by a grant from the U.S. Air Force Office of Scientific Research, and NSF grant CBET-0730359.
4.6 Literature Cited


4.7 List of Figure and Table Captions

Table 4.1: Physiological and morphological characteristics of strain YZ-1 and the most closely phylogenetically related species of the genus *Ochrobactrum*.

Figure 4.1: Schematic diagram (A) and photograph (B) of U-tube MFC (with a carbon cloth anode and a graphite fiber cathode) used for isolation of an exoelectrogenic bacterium.

Figure 4.2: Current generation (normalized to the anode surface area) as a function of time for eight sequential 10-fold dilutions (10^{-1} to 10^{-8}) in eight U-tube MFC reactors using 1 g/L acetate as the sole electron donor for the first cycle of dilution-to-extinction isolation of an exoelectrogenic bacterium. A sterile U-tube system was used as an uninoculated control.

Figure 4.3: DGGE profiles based on 16S rRNA gene from the initial mixed culture and each of the highest cell dilutions capable of electricity production at the end of each cycle (I, initial; cycle 1 to 8 [lanes 1 to 8, respectively]). The bacterial community dynamics indicates that strain YZ-1 (band 1) was isolated through successive cycles.

Figure 4.4: Phylogenetic tree of strain YZ-1 and closely related species based on 16S rRNA gene sequences. The tree was constructed using the neighbor-joining method. The
numbers at nodes indicate the percentages of occurrence of the branching order in 1000 bootstrapped trees for values greater than 50%. Scale bar = 1% divergence.

Figure 4.5: Transmission electron micrograph of strain YZ-1 (A) and scanning electron micrographs of YZ-1 on the anode surface in U-tube MFC reactors fed with 1 g/L acetate as the sole electron donor (B, C and D). (B) Carbon cloth anode surface colonized by YZ-1 with cells attached. (C) Colony of YZ-1 completely covering the anode surface. (D) YZ-1 attached to a single carbon cloth fiber.

Figure 4.6: Power density (filled symbols) and voltage (open symbols) as a function of current density (normalized to the anode area) obtained by varying the external circuit resistance (250 to 5000Ω) for (A) strain YZ-1 and (B) domestic wastewater in U-tube MFCs using 1 g/L acetate. The error bars indicate standard deviations based on averages measured during stable power output in two or more separate batch experiments.

Figure 4.7: CE obtained by varying the external circuit resistance (250 to 5000Ω) for strain YZ-1 and domestic wastewater in U-tube MFCs using 1 g/L acetate. The error bars indicate standard deviations based on averages measured during stable power output in two or more separate batch experiments.

Figure 4.8: Electricity generation (based on the anode surface area) by strain YZ-1 using different carbon sources (all at a concentration of 1 g/L) in U-tube MFC reactors The
error bars indicate standard deviations based on averages measured during stable power output in two or more separate batch experiments.
Table 4.1: Physiological and morphological characteristics of strain YZ-1 and the most closely phylogenetically related species of the genus *Ochrobactrum*.

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*a* the data for *O. anthropi* were obtained from Holmes, et al., 1988 (33); the data for *O. lupini* were obtained from Trujillo et al., 2005 (35), and the data for *O. cytisi* were obtained from Zurdo-Piñerio et al., 2007 (34). +, positive; -, negative; w, weak; ND, not done.

*b* Data from this study for *O. anthropi* ATCC 49188<sup>T</sup> (= LMG 3331<sup>T</sup>), reported as positive by Holmes *et al.*, 1988 (33).
Figure 4.1: Schematic diagram (A) and photograph (B) of U-tube MFC (with a carbon cloth anode and a graphite fiber cathode) used for isolation of an exoelectrogenic bacterium.
Figure 4.2: Current generation (normalized to the anode surface area) as a function of time for eight sequential 10-fold dilutions ($10^{-1}$ to $10^{-8}$) in eight U-tube MFC reactors using 1 g/L acetate as the sole electron donor for the first cycle of dilution-to-extinction isolation of an exoelectrogenic bacterium. A sterile U-tube system was used as an uninoculated control.
Figure 4.3: DGGE profiles based on 16S rRNA gene from the initial mixed culture and each of the highest cell dilutions capable of electricity production at the end of each cycle (I, initial; cycle 1 to 8 [lanes 1 to 8, respectively]). The bacterial community dynamics indicates that strain YZ-1 (band 1) was isolated through successive cycles.
Figure 4.4: Phylogenetic tree of strain YZ-1 and closely related species based on 16S rRNA gene sequences. The tree was constructed using the neighbor-joining method. The numbers at nodes indicate the percentages of occurrence of the branching order in 1000 bootstrapped trees for values greater than 50%. Scale bar = 1% divergence.
Figure 4.5: Transmission electron micrograph of strain YZ-1 (A) and scanning electron micrographs of YZ-1 on the anode surface in U-tube MFC reactors fed with 1 g/L acetate as the sole electron donor (B, C and D). (B) Carbon cloth anode surface colonized by YZ-1 with cells attached. (C) Colony of YZ-1 completely covering the anode surface. (D) YZ-1 attached to a single carbon cloth fiber.
Figure 4.6: Power density (filled symbols) and voltage (open symbols) as a function of current density (normalized to the anode area) obtained by varying the external circuit resistance (250 to 5000Ω) for (A) strain YZ-1 and (B) domestic wastewater in U-tube MFCs using 1 g/L acetate. The error bars indicate standard deviations based on averages measured during stable power output in two or more separate batch experiments.
Figure 4.7: CE obtained by varying the external circuit resistance (250 to 5000Ω) for strain YZ-1 and domestic wastewater in U-tube MFCs using 1 g/L acetate. The error bars indicate standard deviations based on averages measured during stable power output in two or more separate batch experiments.
Figure 4.8: Electricity generation (based on the anode surface area) by strain YZ-1 using different carbon sources (all at a concentration of 1 g/L) in U-tube MFC reactors. The error bars indicate standard deviations based on averages measured during stable power output in two or more separate batch experiments.
Chapter 5

Tubular Membranes Cathodes for Scalable Power Generation in Microbial Fuel Cells

Abstract

One of the greatest challenges for using microbial fuel cells (MFCs) for wastewater treatment is creating a scalable architecture that provides large surface areas for oxygen reduction at the cathode and bacteria growth on the anode. We demonstrate here a scalable cathode concept by showing that a tubular ultrafiltration membrane with a conductive graphite coating and a non-precious metal catalyst (CoTMPP) can be used to produce power in an MFC. Using a carbon paper anode (surface area $A_{an} = 7$ cm$^2$, surface area per reactor volume $A_{an,s} = 25$ m$^2$/m$^3$), an MFC with two 3-cm tube-cathodes ($A_{cat} = 27$ cm$^2$, $A_{cat,s} = 84$ m$^2$/m$^3$) generated up to 8.8 W/m$^3$ (403 mW/m$^2$) using glucose [0.8 g/l in a 50 mM phosphate buffer solution (PBS)], which was only slightly less than that produced using a carbon paper cathode with a Pt catalyst (9.9 W/m$^3$, 394 mW/m$^2$; $A_{cat} = 7$ cm$^2$, $A_{cat,s} = 25$ m$^2$/m$^3$). Coulombic efficiencies (CEs) with carbon paper anodes were 25-40% with tube cathodes (CoTMPP), compared to 7-19% with a carbon paper cathode. When a high-surface-area graphite brush anode was used ($A_{an} = 2235$ cm$^2$, $A_{an,s} = 7700$ m$^2$/m$^3$) with two tube cathodes placed inside the reactor ($A_{cat} = 27$ cm$^2$, $A_{cat,s} = 93$ m$^2$/m$^3$), the MFC produced 17.7 W/m$^3$ with a CE = 70-74% (200 mM PBS). Further increases in
the surface area of the tube-cathodes to 54 cm² (120 m²/m³) increased the total power output (from 0.51 to 0.83 mW), but the increase in volume resulted in a constant volumetric power density (~18 W/m³). These results demonstrate that an MFC design using tubular cathodes coated with nonprecious metal catalysts, and brush anodes, is a promising architecture that is intrinsically scalable for creating larger systems. Further increases in power output will be possible through the development of cathodes with lower internal resistances.³

5.1 Introduction

A microbial fuel cell (MFC) is a promising technology for wastewater treatment because it can produce electricity at the same time that the biodegradable organic matter is removed (1-3). In an MFC system, the characteristics of the cathode can substantially affect electricity generation (4-6). Various catholytes have been used in MFCs, including oxygen (7, 8), ferricyanide (9-11) and metal oxides such as MnO₂ (12). However, oxygen is considered as the most suitable electron acceptor for practical MFC systems because it is free and its use is sustainable (13). Exposing one side of the cathode to air (air cathode) has significantly improved power production, compared to dissolving air in the cathode electrolyte (aqueous cathode) (2). In a single-chamber MFC containing an air cathode, the maximum power density was 262 mW/m² (6.6 W/m³; Coulombic efficiency (CE) = 40 - 55 %) using glucose when a cation exchange membrane (Nafion) was hot-pressed onto the cathode (7). Removing the Nafion membrane increased the power density to 494 mW/m² (12.4 W/m³), but decreased the CE to 9-12 % as a result of greater oxygen diffusion into the reactor (7). Adding a diffusion layer to the air side of the cathode increased the CE to 20-27 %, and also increased power production to 766 mW/m² (19.2 W/m³) (5).

One of the biggest challenges for developing large-scale MFCs is a method to produce a scalable air-cathode architecture that is cost-effective. Tubular cathodes can provide high surface area to volume ratios, but there have been few investigations with this type of architecture in MFCs. In one system a tubular cathode was made by hot-
pressing carbon cloth containing a Pt catalyst to a Nafion membrane, and then wrapping the material around a porous plastic tube. This approach generated 26 mW/m\(^2\) (1.6 W/m\(^3\)) with domestic wastewater as a substrate (14). Two other approaches have been tried, but both used ferricyanide as a catholyte rather than oxygen. In one system the cylindrical cathode made of a woven graphite mat was placed on a cation exchange membrane and wrapped around the anode so that the ferricyanide solution flowed down over the outside of the cathode (15). In the second system the ferricyanide solution was pumped through a cation exchange membrane formed tube, with graphite granules packed into the tube as the cathode material, and then the tube was placed in a concentric manner inside a column containing a bed of graphite granules that served as the anode (16). While these two studies with ferricyanide have achieved greater power production than systems using oxygen, it is generally accepted that the use of ferricyanide is unsustainable and thus not a practical approach for MFC applications such as wastewater treatment (2).

Another limitation of existing air cathode systems is the catalyst. Platinum is widely used in laboratory systems for air cathodes to catalyze oxygen reduction, but it is also considered to be impractical for large scale systems due to its high cost. Co-tetra-methyl phenylporphyrin (CoTMPP) and iron phthalocyanine (FePc) have recently been shown to be suitable alternatives to Pt in MFCs (17, 18).

In this study, we demonstrate a new approach for cathode design based on converting tubular ultrafiltration membranes into MFC cathodes. Instead of joining carbon cloth or paper to a membrane or using graphite granules, we made the membrane itself the cathode by coating it with a high graphite content paint. We then added CoTMPP to the painted surface to catalyze the reduction of oxygen to water. The use of a
tube cathode permits passive transport of oxygen to the catalyst, while at the same time limiting water loss due to the low water membrane permeability but allowing proton transport to the air-facing side of the membrane. The performance of various types of tube cathodes was investigated using conventional carbon paper anodes and newly developed graphite brush anodes described in a companion paper in this issue (19). We compared the performance of these systems to that of a commercial carbon paper cathode in terms of power and CE, and examined different locations of the catalyst (inside and outside of the tube), configurations of the cathode (inside and outside the reactor), and operation mode of the reactor (fed batch and continuous flow).

5.2 Methods

5.2.1 Cathode Preparation

An ultrafiltration hydrophilic tubular membrane (a polysulfone membrane on a composite polyester carrier) with an inner diameter of 14.4 mm (B0125, X-FLOW) and wall thickness of 0.6 mm was used as the tube-cathode. The tubes were cut to lengths of 3, 6 or 12 cm (equal to a surface area of 13.5, 27, and 54 cm²) and then were coated with a proprietary graphite paint two times (ELC E34 Semi-Colloidal, Superior Graphite Co.). Co-tetra-methylphenylporphyrin (CoTMPP) was used as the cathode catalyst unless indicated otherwise. A CoTMPP/carbon mixture (20% CoTMPP) was prepared as previously described (17) and mixed with a 5% Nafion solution to form a paste (7 µl of Nafion per mg of CoTMPP/C catalyst). The paste was then applied to the air-facing
surfaces of all tube-cathodes (~0.5 mg/cm² CoTMPP loading). In some tests a commercial carbon paper cathode containing Pt (0.35 mg/cm² of Pt catalyst, water proofed paper, E-Tek; $A_{cat} = 7$ cm²) was used with the catalyst facing the water solution. A 3-cm tube-cathode containing only graphite paint was prepared as a noncatalyst control.

5.2.2 Anode Preparation

The anode electrode was either a piece of plain Toray carbon paper (without wet proofing; E-Tek; $A_{an} = 7$ cm²) or a plain graphite fiber brush (25 mm diameter × 25 mm length; fiber type: PANEX® 33 160K, ZOLTEK) with an estimated surface area of 2235 cm² (95% porosity) (19).

5.2.3 Tube Cathode Reactors with Carbon Paper Anodes

In order to distinguish among different types of reactors, we designated each reactor configuration using the notation of X-YZ-J, where: X = anode material (C = carbon paper, B = graphite brush); Y = cathode material (C = carbon paper, Tn = number of 3-cm lengths of tube cathodes, where $n = 1 - 4$); Z = catalyst (Pt = platinum; Co = CoTMPP; C = carbon without catalysts); and J = cathode configuration (I = inside reactor, O = outside reactor) (Figure 5.1). Three single-chamber carbon paper anode (C) MFCs were constructed with the tube-cathodes located inside (I) cylindrical chambered reactors (4 or 6 cm length × 3 cm diameter) (Table 5.1). Two of these reactors were constructed with CoTMPP coated tube-cathodes (TCo). One had a single 3-cm tube (C-T1Co-I; 4-cm
chamber), for a total cathode surface area of $A_{cat} = 13.5 \text{ cm}^2$, and a surface area normalized to the reactor volume of $A_{cat,s} = 59 \text{ m}^2/\text{m}^3$, while the other had two 3-cm tubes connected by a wire ($\text{C-T}_2\text{Co-I}; 6$-cm chamber; $A_{cat} = 27 \text{ cm}^2$, $A_{cat,s} = 84 \text{ m}^2/\text{m}^3$) (Figure 5.1B and C). The third system contained a single 3-cm tube-cathode without any catalyst ($\text{C-T}_1\text{C-I}; 4$-cm chamber; $A_{cat} = 13.5 \text{ cm}^2$, $A_{cat,s} = 59 \text{ m}^2/\text{m}^3$) (Figure 5.1B). Each cathode tube was inserted through the center of a single 2-cm-long slice of the chamber, with the carbon paper anode placed at an opposite side of another 2-cm-long slice. The CoTMPP catalyst layer was coated on the inside of these tubes (membrane side) and faced air. A single-chamber cube MFC of same type used in previous studies (7) was also tested by using a carbon paper anode and a carbon paper cathode with a Pt catalyst ($\text{C-CPt-I}; A_{cat} = 7\text{ cm}^2$, $A_{cat,s} = 25 \text{ m}^2/\text{m}^3$), with the electrodes placed at opposite sides of the chamber (4 cm length $\times$ 3 cm diameter) (Figure 5.1A).

5.2.4 Tube-Cathode Reactors with Brush Anodes

Two different brush anode (B) MFC configurations were tested with tube cathodes containing a CoTMPP catalyst (TCo): a cylindrical chambered MFC (6 cm long $\times$ 3 cm diameter) with tubes inside (I) the reactor ($\text{B-T}_2\text{Co-I}$); and the same type of reactor (4 cm $\times$ 3 cm diameter), but with the tube cathode placed outside (O) the reactor ($\text{B-T}_2\text{Co-O}$) (Table 5.1). For the inside tube reactor, a graphite brush anode placed vertically in a 2-cm-long reactor slide, and two wire-connected tube cathodes each 3 cm long were inserted through adjacent 2-cm slices producing a 6-cm long reactor ($\text{B-T}_2\text{Co-I}; A_{cat} = 27 \text{ cm}^2$, $A_{cat,s} = 93 \text{ m}^2/\text{m}^3$) (Figure 5.1E). The catalyst was coated on the inside of
the tube (membrane side) and faced the air. The MFC with the cathode tube placed outside of the cube reactor was constructed using a brush anode placed horizontally in the center of a 4-cm-long chamber, with a single 6-cm-long (2 × 3 cm) cathode tube extending from one side of the chamber (B-T₂Co-O; \(A_{\text{cat}} = 27 \text{ cm}^2\), \(A_{\text{cat,s}} = 75 \text{ m}^2/\text{m}^3\)) (Figure 5.1D). In this case, the catalyst was coated on the outside of the tube (supporting side of membrane) and faced the air.

To further investigate the effect of cathode surface area, additional 3-cm tube cathodes were added to the inside of the MFCs, with external wires connecting the tubes (B-T₃Co-I and B-T₄Co-I). For reactors with tubes outside the reactor, the tube length was increased to 12 (4 × 3) cm (B-T₄Co-O), producing a cathode surface area of 54 cm².

5.2.5 Start Up and Operation

All MFCs were inoculated with a 50:50 mixture of domestic wastewater (~300 mg COD/L) and glucose (0.8 g/L) in phosphate buffer solution (PBS, 50 mM; pH=7.0) in a nutrient medium (7). After 2-3 repeated feeding cycles, only media (no wastewater) was added. Reactors were considered to be acclimated if the maximum voltage produced was repeatable for at least three batch cycles. Following these tests, brush anode reactors were switched to 200 mM PBS as solution conductivity has been shown to increase power generation (20, 21). The medium in the reactor was refilled when the voltage dropped below ~20 mV (resistances of 40-500 Ω) or ~40 mV (1000-3000 Ω).

Reactors with brush anodes and tube cathodes placed inside or outside the reactor were also operated in continuous flow mode with a hydraulic retention time (HRT) of 24
hours (total volume of reactor). The influent was fed from the anode side (Figure 5.1D and E) by using a micro-infusion pump (AVI micro 210A infusion pump, 3M), with the flow discharged from the cathode side. All experiments were performed at 30°C.

5.2.6 Calculations and Measurements

The Voltage ($V$) output of all reactors were measured across a fixed external resistance (1000 Ω except as noted) using a data acquisition system (2700, Keithly, USA). Electrode potentials were measured using a multimeter (83 III, Fluke, UAS) and a reference electrode (Ag/AgCl; RE-5B, Bioanalytical systems, USA). Current ($I = V/R$), power ($P = IV$), and CE (based on the input glucose) were calculated as previously described (22). Power and current density were either normalized to the projected area of carbon paper anodes ($m^2$) or the total reactor volume ($m^3$). To obtain the polarization curve and power density curve as a function of current, external circuit resistances were varied from 40 to 3000 Ω. For batch tests, one resistor was used for a full cycle (at least 24 hours) for at least two separate cycles, while for continuous flow tests at least 24 hours was used for each resistor.

Internal resistance, $R_{int}$, was measured by electrochemical impedance spectroscopy (EIS) over a frequency range of $10^5$ to 0.005 Hz with sinusoidal perturbation of 10 mV amplitude using a potentiostat (PC 4/750 potentiostat, Gamry Instrument Inc.) for carbon paper anode MFCs filled with a nutrient media containing 50 mM PBS and brush anode reactors using 200 mM PBS. The anode was used as the working electrode and the cathode as the counter and reference electrode as described previously (23).
The maximum rate of oxygen transfer through a tube cathode was determined by measuring oxygen accumulation in an uninoculated carbon paper anode MFC reactor containing a clean 3-cm tubular membrane (without any graphite/catalysts) and deoxygenated deionized water. The effective oxygen mass transfer coefficient of $k$ was determined as previously described (5) with a dissolved oxygen probe (Foxy-21G, Ocean Optics Inc., FL) placed at the centre of the stirred reactor. The resistance of proton transport through the tubular membrane cathode was determined by measuring the internal resistance increase when adding this membrane material between two carbon electrodes in a two-chamber cube reactor as previously described (24). The membrane tube was sliced open, cut into a circular shape to produce a flat surface of 7 cm$^2$, and then placed in the middle of the reactor with carbon electrodes each spaced 2 cm from the membrane. The internal resistances of the reactor with the membrane ($R_{int,m+}$) and without any membrane ($R_{int,m-}$) were measured by EIS using a potentiostat. The proton transport resistivity (Ω·cm$^2$) of the tubular membrane was calculated as $(R_{int,m+} - R_{int,m-}) \times A_{mem}$.

COD concentrations of the reactor effluent were measured using standard methods (25).
5.3 Results

5.3.1 Power Production from Tube Reactors with Carbon Paper Anodes

Repeatable cycles of power production were rapidly generated after acclimation of all four carbon paper anode MFC reactors. Power density curves and polarization curves obtained by varying the external circuit resistances from 40 to 3000 Ω showed that the tube cathode MFC with two CoTMPP coated tubes (C-T2Co-I; \( A_{cat} = 27 \text{ cm}^2 \)) produced power only somewhat less than that achieved with a carbon paper cathode with Pt catalyst (C-CPt-I; \( A_{cat} = 7 \text{ cm}^2 \)), with a maximum power density of \( 8.8 \pm 1.0 \text{ W/m}^3 \) (403 ± 33 mW/m², anode surface area) for the tube cathode system and \( 9.9 \pm 0.1 \text{ W/m}^3 \) (394 ± 3 mW/m²) for the carbon paper cathode (both at \( R_{ext} = 250 \Omega \); Figure 5.2A). Decreasing the tube cathode area by 50% (C-T1Co-I, \( A_{cat} = 13.5 \text{ cm}^2 \)) slightly affected the volumetric power density (9.3 ± 0.3 W/m³; \( R_{ext} = 250 \Omega \)) due to the reduced volume without the cathode, but reduced power by 24% on the basis of the anode surface area (306 ± 8 mW/m²). In the absence of a catalyst, the tube reactor (C-T1C-I, \( A_{cat} = 13.5 \text{ cm}^2 \)) produced much less power, or 3.1 ± 0.1 W/m³ (\( R_{ext} = 250 \Omega \)) (Figure 5.2A). The internal resistances of these four MFCs ranged from 84 to 131 Ω (Table 5.1).

As expected, all carbon paper anode MFCs had similar anode potentials at the same current (Figure 5.2B). The differences in power productions from these four MFC reactors were a result of the differences in cathode potentials. Tube cathode potentials were improved by adding CoTMPP as the catalyst and/or increasing the cathode surface area. With 13.5 or 27 cm² of surface area, the CoTMPP coated tube-cathodes (C-T1Co-I
and C-T₂Co-I) achieved almost same potentials as the carbon paper Pt cathode (C-CPt-I) over the current density range of 0-60 A/m³.

### 5.3.2 Power Production from Tube Reactors with Brush Anodes

All of the tube reactors with brush anodes generated repeatable power cycles after ~14 batch cycles (50 mM PBS). After the buffer concentration was increased to 200 mM, a maximum volumetric power density of 17.7 ± 0.2 W/m³ \((R_{ext} = 250 \, \Omega)\) was produced with two 3-cm tube cathodes inside the reactor (B-T₂Co-I, \(A_{\text{cat}} = 27 \, \text{cm}^2\)) (Figure 5.3A). The 200% increased power produced with the brush versus the carbon paper anode in the same type of tube cathode reactor (C-T₂Co-I, 8.8 ± 1.0 W/m³) was consistent with an overall reduction in internal resistance (from 89 to 66 Ω) and a significant increase of the anode area (from 7 to 2235 cm²). The power produced with brush anode and tube-cathodes inside the reactor was also double the maximum power of 8.2 ± 0.2 W/m³ \((R_{ext} = 250 \, \Omega)\) from the brush reactor with a single 6-cm tube placed outside (B-T₂Co-O, \(A_{\text{cat}} = 27 \, \text{cm}^2\)) (Figure 5.3A). The increase in power output with the tubes inside the reactor was caused by the higher cathode potentials as the brush anode potentials remained unchanged over a current range of 0-58 A/m³ (Figure 5.3B). The OCP of the cathode when inside the reactor (250 ± 8 mV, vs Ag/AgCl) was 112 mV higher than when it was placed outside the reactor (138 ± 16 mV). As the current increased, the potential difference further increased to 240 mV at 58 A/m³ (Figure 5.3B).
5.3.3 Coulombic Efficiencies Using Tube Cathodes

The CEs of all reactors were a function of current densities (Table 1; additional information in Appendix Figure 5A.1). With carbon paper anodes, the tube cathodes with a CoTMPP catalyst achieved CEs as high as 40 %, while carbon paper cathodes with Pt (C-CPt-I) had CEs of 7-19%. Without a catalyst (C-T\textsubscript{1}C-I), the CEs for the tube cathode reactor ranged from 18 to 22%. By using a graphite brush anode, and increasing the solution ionic strength using 200 mM PBS, the CE further increased to 52-58% when the tube was placed outside the reactor (B-T\textsubscript{2}Co-O), and 70-74% for the tube inside one (B-T\textsubscript{2}Co-I).

The higher CEs obtained with tube cathode reactors were thought to be due to lower O\textsubscript{2} diffusion rates through the tubular ultrafiltration membrane than through the carbon paper cathode. For a clean tubular membrane, we measured an O\textsubscript{2} mass transfer coefficient $k = 7.8 \times 10^{-5}$ cm/s, which could result in as much oxygen transfer as 0.03 mgO\textsubscript{2}/h into an MFC system with a tube cathode surface area of 13.5 cm\textsuperscript{2} (C-T\textsubscript{1}Co-I and C-T\textsubscript{1}C-I), or 0.06 mgO\textsubscript{2}/h for a surface area of 27 cm\textsuperscript{2} (C-T\textsubscript{2}Co-I, B-T\textsubscript{2}Co-I and B-T\textsubscript{2}Co-O). In contrast, a carbon paper cathode of 7 cm\textsuperscript{2} (C-CPt-I) produced an oxygen rate of 0.187 mg/h (7). It therefore seems likely that the higher CEs of the tube cathode system were due to the reduction in substrate lost to aerobic oxidation supported by oxygen diffusion through the cathode.
5.3.4 Effect of Tube Cathode Surface Area

The effect of tube-cathode surface area was investigated for brush anode reactors with the tube cathodes placed inside or outside the reactor. The cathode surface areas for both configurations were increased from 27 (T2) to 40.5 (T3) or 54 cm² (T4), by adding more 3-cm tubes inside the reactor (B-T3Co-I and B-T4Co-I) or extending the length of the outside tube up to 12 cm (B-T4Co-O). For the tubes inside the reactor, the maximum power output increased with cathode surface area, producing 0.51 mW (B-T2Co-I), 0.66 mW (B-T3Co-I) and 0.83 mW (B-T4Co-I) (Figure 5.4A). Since the reactor volume also increased by 8 ml when adding each 3-cm tube, however, the volumetric power densities produced by these different reactors with the tubes inside the reactor were similar when normalized to volume, producing for all cases a maximum of ~18 W/m³ (Figure 5.4B). When the tube was placed outside the reactor, the maximum power output was not improved with increased tube length (Figure 5.4A). Although both reactors produced ~0.3 mW, the longer tube cathode added 10 ml more volume than the shorter one, resulting in a decrease in volumetric power from 8.2 (B-T2Co-O) to 6.5 W/m³ (B-T4Co-O) (Figure 5.4B).

5.3.5 Continuous Flow Performance of Tube Cathode Reactors

Two brush anode MFCs with the tube cathodes inside or outside the reactor were also operated in continuous flow mode. With the tubes inside the reactor (B-T2Co-I; \( A_{\text{cat}} = 27 \text{ cm}^2 \)), the voltage output (520 mV at 1000 \( \Omega \)) was immediately produced and was stable for more than 10 HRTs (Figure 5.5A). Power density curves showed that the
performance was identical to that produced in fed-batch tests, resulting in a maximum power density of \(~18 \text{ W/m}^3\) (Figure 5.5B).

Power density curves measured for the MFC with the tube outside the reactor were also similar for continuous and fed batch operation (Figure 5.5B). However, the voltage produced by this reactor (B-T$_2$Co-O, $A_{\text{cat}} = 27 \text{cm}^2$) was unstable over time, and decreased from 500 to 380 mV (1000 $\Omega$) (Figure 5.5A). Over time, the catalyst layer coating on the outside of the tube gradually cracked, with visible salt accumulation on the outside of the tube. On average, the system could operate for only 100 hours before the catalyst layer showed large cracks.

The effluents from both reactors operated in continuous flow mode were analyzed with a fixed external resistor of 1000 $\Omega$. The reactor with the tube outside the MFC produced a COD removal of $53 \pm 5 \%$, compared to $37 \pm 5 \%$ when the tubes were inside the reactor.

### 5.3.6 Internal Resistance Contributed by Tube Cathodes

The internal resistance with a flat piece of tubular membrane material (7 cm$^2$) placed between two carbon electrodes in a two-chamber cube reactor, was measured as $R_{\text{int,}m^+} = 247 \pm 6 \Omega$. When the membrane was removed, the internal resistance was $R_{\text{int,}m^-} = 84 \pm 1 \Omega$, which was the same as reported by Kim et al (24). These resistances indicate that the proton transport resistivity of the membrane was $1141 \text{ \Omega cm}^2$, resulting in internal resistances of $84 \Omega$ or $42 \Omega$ for the 13.5 cm$^2$ or 27 cm$^2$ tubular membrane.
cathodes. This indicates that the membrane accounted for up to 64% of the total internal resistances of the tube cathode reactors.

5.4 Discussion

Coating tubular ultrafiltration membranes with a graphite paint and a nonprecious metal catalyst (CoTMPP) produced a maximum volumetric power density of 17.7 W/m³, while at the same time achieving a CE of 70-74% when the cathode tube membranes were placed inside the reactor and close to the brush anode (B-T₂Co-I). This volumetric power density was greater than that produced here with a cube reactor using two carbon paper electrodes (9.9 W/m³) and 4-cm spacing between the electrodes. The volumetric power density produced with the tube cathodes was also larger than that produced with a same type of cube reactor when a Nafion membrane was hot-pressed to a carbon cloth cathode reported by Liu et al. (262 mW/m², 6.6 W/m³) (7). Higher power densities by tube cathode reactors were limited by the reactor internal resistance, which was measured to range from 66 to 131 Ω. Internal resistances using ferricyanide cathode MFC systems have been equal to or lower than these values, ranging from 4-8 to 84 Ω, and resulting in power volumetric densities of 38 W/m³ (with glucose) and 48 W/m³ (with acetate) (15) to 3.1W/m³ (with a sucrose and yeast-extract solution) (26).

Increasing power production in a tubular cathode MFC will require the development of tubular membranes that have lower internal resistances. It was recently shown by Kim et al. (24) that a Nafion membrane did not affect the internal resistance when it was placed in solution between the anode and cathode in a same type of two-
chamber cube reactor used in this study. Thus, it is not the presence of the Nafion membrane per se that affected power production in the study by Liu et al. (7), but rather a result of the bonding of the membrane to the cathode. It was also shown by Kim et al. (24) that replacing the Nafion membrane with an ultrafiltration (UF) membrane significantly increased the internal resistance. Adding the UF tubular membrane used here between electrodes in that cube reactor increased the internal resistance to $247 \, \Omega$, compared to $84 \, \Omega$ produced without a membrane or when using a Nafion membrane (24) in the same system. These results showed that the tubular membrane cathodes contributed $42 \, \Omega$ ($27 \, \text{cm}^2$ membrane area) of the $66 \, \Omega$ for the brush anode MFC with two tube cathodes inside the reactor and filled with $200 \, \text{mM PBS}$, or $64 \%$ of the total internal resistance. Other types of flat UF membranes examined by Kim et al. (24) produced internal resistances of $1814$, $98$ and $91 \, \Omega$ for molecular weight cut offs of $0.5$, $1$ and $3 \, \text{K}$. In contrast, cation and anion exchange membranes produced $84$ and $88 \, \Omega$ resistances under the same conditions (24). These findings indicate that it will be possible to substantially improve reactor performance by developing tubular membranes that have lower internal resistances than those tested here.

The architecture of the MFC containing tube membranes will be an important factor in power generation. Comparisons of power production with the cathode tubes in different orientations (inside and outside the reactor), and with flow directed to the cathode in different ways (around the tube versus through the tube) suggests that the best anode brush and cathode tube orientation is with the brush and tube placed as closely as possible in the same chamber. The potential of the cathode with the tubes placed inside reactor was $112-240 \, \text{mV}$ higher than when the tube was outside the reactor (current range
of 0–58 A/m$^3$). This higher voltage resulted for several reasons. First, with the tubes inside the reactor (B-T$_2$Co-I) there was a shorter distance between the electrodes (3 cm vs 5 cm, measured between the anode and the midpoint of the tube cathode), producing a lower internal resistance ($66 \pm 1 \Omega$ versus $85 \pm 8 \Omega$), which increased the cathode potential as previously observed (20). Second, when the tubes were on the inside of the reactor the catalyst was on the membrane side (polysulfone) of the tube, versus the supporting side (polyester). This difference in location could alter the effectiveness of the catalyst in terms of proton and oxygen diffusion to the catalyst surface on the supported material. Finally, we observed that when the reactor was operated in continuous flow, with the water flow was directed through the tube (B-T$_2$Co-O), power production was not stable. Under these conditions, the catalyst layer cracked and we observed extensive salt precipitation on the outside of the tube, indicating this within-tube orientation of the flow as not a suitable reactor configuration.

The specific surface areas of the two electrodes per volume of reactor (specific surface areas) will need to be optimized. Previous findings with brush anodes suggests that anode specific surface areas in the range used here are well in excess of that needed for maximum power production (19). However, the same is not true for cathode surface areas as tests conducted here showed increasing cathode surface area increased power. Increasing the cathode surface area from 27 up to 54 cm$^2$ (from 93 to 120 m$^2$/m$^3$; B-T$_2$Co-I, B-T$_3$Co-I, B-T$_4$Co-I) increased the power generation by up to 63%. However, because of the large size of the cathode tubes, the power normalized to reactor volume was essentially unchanged due to the increase in reactor volume needed to accommodate the large cathodes. It was not possible with the cathode tubes used here to maintain a
constant reactor volume, but in future tests with larger reactors this may be possible. In other systems this effect of cathode surface area on power has also been observed. For example, in two-bottle MFCs, it was observed that increasing the cathode surface area from 22.5 to 67.5 cm\(^2\) (from 9 to 27 m\(^2/m^3\)) increased power output by 24% (27). An MFC used to generate power from domestic wastewater produced only 1.6 W/m\(^3\) (26 mW/m\(^2\)), but that reactor had a cathode specific surface area of only 30 m\(^2/m^3\). These specific surface areas so far tested for air cathode MFCs are well below surface areas used in membrane bioreactors of 180 to 6800 m\(^2/m^3\) (28, 29). Thus, it is likely MFC reactor performance can be increased using membranes with larger surface area to volume ratios. However, maximizing the cathode surface area is not the only challenge as membrane cost must also be considered. The tubular membranes we used here cost $350/m\(^2\), which is less expensive than Nafion ($1400/m\(^2\)), but more expensive than other types of commercially available membranes. Thus the relative amounts of anode and cathode materials will need to be optimized based on cost and performance.

This procedure of converting conventional nonconductive ultrafiltration membranes into electrically conductive and catalytically active cathodes opens up a whole new approach towards MFC design, and it should allow easy scale up of the system when combined with the high specific surface areas of graphite brush anodes (30). Different types of membranes and other modifications to the conductive materials can be expected to increase power. The CoTMPP catalyst used here is indicated to be suitable for power generation, but other nonprecious catalysts should be investigated for performance and longevity in these systems. In addition, it has been recently shown by our group that a high-temperature ammonia treatment of the brush electrode can increase
power production and reduce acclimation time of the MFC for power production (19). These and other anode treatments (31-33) could therefore be expected to improve performance of subsequent systems. The high recovery of electrons with the tube cathodes, as indicated by high CEs, the use of readily available materials, reasonable costs of these materials, and promising performance in these designs all indicate that MFCs based on these brush anodes and tube cathodes are now ready for the next level of testing in larger systems. Through the analysis of performance of these larger systems, it will be possible to evaluate the performance of the system for use as a wastewater treatment technology.

5.5 Acknowledgments

The authors thank D.W. Jones for help with analytical measurements and NORIT Process Technology B.V. and X-flow B.V. (Netherlands) for providing the membranes. This research was supported by National Science Foundation Grant BES-0401885.
5.6 Literature Cited


5.7 List of Figure and Table Captions

Table 5.1: Electrode types and surface areas, ratios of electrode area to volume, volumes, internal resistances, maximum power density normalized to anode surface area or total reactor volume, and CEs for all carbon paper and brush anode MFC batch tests. (See text for reactor designation codes)

Figure 5.1: Different MFC configurations. Carbon paper anode MFCs with (A) Pt on a carbon paper cathode (C-CPt-I), (B) 3-cm tube-cathode (C-T1Co-I or C-T1C-I), and (C) two 3-cm tube-cathodes (C-T2Co-I). Brush anode MFCs with (D) a single 6-cm long tube-cathode outside the reactor (B-T2Co-O), and (E) two 3-cm tube-cathodes placed inside the MFC (B-T2Co-I).

Figure 5.2: (A) Power density (open symbols), voltage (filled symbols) and (B) electrode potentials (cathode open symbols, anode filled symbols) as a function of current density (normalized to total reactor volume) obtained by varying the external circuit resistance (40–3000Ω) for carbon paper anode MFCs.

Figure 5.3: (A) Power density (open symbols), voltage (filled symbols) and (B) electrode potentials (cathode open symbols, anode filled symbols) as a function of current density (based on reactor volume) obtained by varying the external circuit resistance (40–3000Ω) for brush anode MFCs.
Figure 5.4: (A) Power and (B) volumetric power density as a function of the cathode surface area of tube cathode MFCs with brush anodes.

Figure 5.5: (A) Voltage as a function of time at a fixed resistance of 1000 Ω (except as noted) and (B) volumetric power density as a function of current (normalized to volume) obtained by varying the external circuit resistance (40–3000Ω) for brush anode MFCs operated in continuous or batch mode. Vertical lines indicate where the external resistance was changed for polarization curve measurements. Arrows indicate the replacement of the tube cathode outside the reactor.
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<table>
<thead>
<tr>
<th>Anode material</th>
<th>Cathode material</th>
<th>Cathode catalyst</th>
<th>Catalyst location</th>
<th>Reactor designation</th>
<th>$A_{an}$ (cm²)</th>
<th>$A_{an,s}$ (m²/m³)</th>
<th>$A_{cat}$ (cm²)</th>
<th>$A_{cat,s}$ (m²/m³)</th>
<th>Volume (ml)</th>
<th>$R_{int}$ (Ω)</th>
<th>$P_{max}$ (mW/m²)(^a)</th>
<th>$P_{max}$ (W/m³)</th>
<th>CE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon paper</td>
<td>2 tubes (3 cm each)</td>
<td>CoTMPP</td>
<td>Inside tube</td>
<td>C-T₂Co-I</td>
<td>7</td>
<td>22</td>
<td>27</td>
<td>84</td>
<td>32</td>
<td>89 ± 5</td>
<td>403 ± 33</td>
<td>8.8 ± 1.0</td>
<td>25-33</td>
</tr>
<tr>
<td>Carbon paper</td>
<td>1 tube (3 cm each)</td>
<td>CoTMPP</td>
<td>Inside tube</td>
<td>C-T₁Co-I</td>
<td>7</td>
<td>30</td>
<td>13.5</td>
<td>59</td>
<td>23</td>
<td>131 ± 5</td>
<td>306 ± 8</td>
<td>9.3 ± 0.3</td>
<td>31-40</td>
</tr>
<tr>
<td>Carbon paper</td>
<td>1 tube (3 cm each)</td>
<td>None</td>
<td>Inside tube</td>
<td>C-T₁C-I</td>
<td>7</td>
<td>30</td>
<td>13.5</td>
<td>59</td>
<td>23</td>
<td>131 ± 5</td>
<td>101 ± 2</td>
<td>3.1 ± 0.1</td>
<td>18-22</td>
</tr>
<tr>
<td>Carbon paper</td>
<td>Carbon paper</td>
<td>Pt</td>
<td>Inside reactor</td>
<td>C-CPt-I</td>
<td>7</td>
<td>25</td>
<td>7</td>
<td>25</td>
<td>28</td>
<td>84 ± 1</td>
<td>394 ± 3</td>
<td>9.9 ± 0.1</td>
<td>7-19</td>
</tr>
<tr>
<td>Graphite brush</td>
<td>2 tubes (3 cm each)</td>
<td>CoTMPP</td>
<td>Inside tube</td>
<td>B-T₂Co-I</td>
<td>2235</td>
<td>7700</td>
<td>27</td>
<td>93</td>
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<td>66 ± 1</td>
<td>$b$</td>
<td>17.7 ± 0.2</td>
<td>70-74</td>
</tr>
<tr>
<td>Graphite brush</td>
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<td>CoTMPP</td>
<td>Outside tube</td>
<td>B-T₂Co-O</td>
<td>2235</td>
<td>6200</td>
<td>27</td>
<td>75</td>
<td>36</td>
<td>85 ± 8</td>
<td>$b$</td>
<td>8.2 ± 0.2</td>
<td>52-58</td>
</tr>
</tbody>
</table>

\(^a\)Normalized to projected surface area of planar anodes. \(^b\)Not applicable.
Figure 5.1: Different MFC configurations. Carbon paper anode MFCs with (A) Pt on a carbon paper cathode (C-CPt-I), (B) 3-cm tube-cathode (C-T1Co-I or C-T1C-I), and (C) two 3-cm tube-cathodes (C-T2Co-I). Brush anode MFCs with (D) a single 6-cm long tube-cathode outside the reactor (B-T2Co-O), and (E) two 3-cm tube-cathodes placed inside the MFC (B-T2Co-I).
Figure 5.2: (A) Power density (open symbols), voltage (filled symbols) and (B) electrode potentials (cathode open symbols, anode filled symbols) as a function of current density (normalized to total reactor volume) obtained by varying the external circuit resistance (40 – 3000Ω) for carbon paper anode MFCs. (Error bars ±S.D. based on averages measured during stable power output in two or more separate batch experiments).
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Figure 5.5: (A) Voltage as a function of time at a fixed resistance of 1000 Ω (except as noted) and (B) volumetric power density as a function of current (normalized to volume) obtained by varying the external circuit resistance (40–3000 Ω) for brush anode MFCs operated in continuous or batch mode. Vertical lines indicate where the external resistance was changed for polarization curve measurements. Arrows indicate the replacement of the tube cathode outside the reactor.
Figure 5A.1: Coulombic efficiencies (CEs) as a function of current density (normalized to reactor volume) obtained by varying the external resistance (40-1000 Ω) for carbon paper and brush anode MFCs.
Abstract

One of the main challenges for using microbial fuel cells (MFCs) is developing materials and architectures that are economical and generate high power densities. The performance of two cathodes constructed from two low-cost anion (AEM) and cation (CEM) exchange membranes was compared to that achieved using an ultrafiltration (UF) cathode, when the membranes were made electrically conductive using graphite paint and a non-precious metal catalyst (CoTMPP). The best performance in single-chamber MFCs using graphite fiber brush anodes was achieved using an AEM cathode with the conductive coating facing the solution, at a catalyst loading of 0.5 mg/cm² CoTMPP. The maximum power density was 449 mW/m² (normalized to the projected cathode surface area) or 13.1 W/m³ (total reactor volume), with a Coulombic efficiency up to 70% in a 50 mM phosphate buffer solution (PBS) using acetate. Decreasing the CoTMPP loading by 40 - 80% reduced power by 28 - 56%, with only 16% of the power (72 mW/m²) generated using an AEM cathode lacking a catalyst. Using a current collector (a stainless steel mesh) pressed against the inside surface of the AEM cathode, and 200 mM PBS, the maximum power produced was further increased to 728 mW/m² (21.2 W/m³). The use of AEM cathodes and brush anodes provides comparable performance to similar systems that use materials costing nearly an order of magnitude more (carbon paper electrodes),
and thus represent more useful materials for reducing the costs of MFCs for wastewater treatment applications.\textsuperscript{4}

6.1 Introduction

A microbial fuel cell (MFC) is a new technology for bioenergy production since it can be used to directly produce electricity from the degradation of organic matter contained in various wastes and wastewaters (1-3). Diverse organic substrates have been used as electron donors to produce electricity, including pure chemicals (e.g. fatty acids, alcohols, sugars, proteins, and cellulose [4-7]), complex wastes and wastewaters such as agricultural waste (8) and domestic wastewater (9,10), and other types of wastes and wastewaters (11). Various catholytes, including oxygen (12, 13), ferricyanide (14, 15), ferric iron (16), manganese (17), and permanganate (18), have been used as terminal electron acceptors in MFCs. However, in order to make MFCs competitive with other technologies in renewable energy production, wastewaters and oxygen are considered as the most promising electron donors and acceptor for MFC systems because they are free and sustainable (1, 19).

To develop practical MFC applications for the treatment of wastewater, one of the biggest challenges is to identify low-cost and highly-efficient materials that provide large surface areas needed for bacteria adhesion or oxygen reduction, in ways that allow for a scalable architecture. Carbon paper or cloth is commonly used in MFCs as electrode materials, with Platinum (Pt) as a catalyst on the cathode. While these materials are effective at producing power, they are expensive. Carbon cloth, for example, costs $1000/m² with $140 - $700/m² for the Pt catalyst (0.1 - 0.5 mg-Pt/cm²). A cubic air-cathode MFC containing two flat carbon electrodes (7 cm², 25 m²/m³, 4-cm spacing
between electrodes, Pt catalyst) can be used to produce 494 - 506 mW/m² (12.4 - 12.7 W/m³; no membrane or separator) with glucose or acetate as the substrate (50 mM phosphate buffer solution; PBS) (12, 20). Reducing the electrode spacing from 4 cm to 2 cm increases the power to 811 - 1210 mW/m² (41 - 61 W/m³; 21, 22). Doubling the cathode surface area by using two cathodes (one on each side of the reactor with a ~0.5-cm spacing between the two cathodes), and using a cloth separator to reduce oxygen diffusion to the anode can increase volumetric power density to 627 W/m³ (1120 mW/m²) for fed-batch conditions, and to 1010 W/m³ (1800 mW/m²) under continuous flow operation (23). However, these carbon electrode materials, with a surface area of 560 m²/m³ (for a double-cathode MFC), would have a prohibitive cost for large scale applications.

Materials other than carbon cloth can be used for MFC applications, such as graphite granules, granular activated carbon (GAC), and graphite fiber brush electrodes. These materials can achieve higher specific areas in a scalable architecture (15, 24-27). Graphite granules, with specific surface areas of 817 to 2720 m²/m³ and a porosity of 0.53, were used as the anode material in a tubular packed-bed reactor, producing 48 W/m³ with acetate and 38 W/m³ with glucose (24). When this material was used for both the anode and cathode, a 6-cell stacked MFC generated 258 W/m³ using acetate (15). Using GAC, He et al. (26) produced 29 W/m³ with a sucrose solution. However, a non-sustainable chemical (ferricyanide) was used as a catholyte in all of these graphite granule and GAC studies (15, 24, 26). Brush anodes can provide both high surface area and high porosity, and are less expensive than carbon paper on a surface-area basis. For example, graphite fiber brush can have specific surface areas of 7170 - 18,200 m²/m³ and
porosities of 95 - 98%, at a cost of $0.58/m^2 (based on the surface area of graphite fibers) (27). Using a brush electrode (18,200 m^2/m^3) in a cubic air-cathode MFC, the highest power density achieved was 73 W/m^3 or 2400 mW/m^2 (based on the cathode projected surface area) (27). The optimum amount of graphite fibers needed for these brush electrodes has not yet been optimized, and the cathode remains the greatest challenge for MFC designs.

Tubular membrane cathodes provide a useful method for achieving high surface areas needed when using oxygen as the electron acceptor. Membrane bioreactors used for wastewater treatment are routinely assembled into modules that produce high specific surface areas of 6800 m^2/m^3 (28, 29). It was recently shown that by coating a tubular ultrafiltration (UF) membrane with a conductive graphite paint, and using a non-precious metal catalyst (Co-tetra-methyl phenylporphyrin; CoTMPP), that 18 W/m^3 could be produced in an MFC at a Coulombic efficiency (CE) of 70 - 74% (30). This reactor contained a brush anode (7700 m^2/m^3) and two tubular ultrafiltration (UF) membrane cathodes (UF-B0125, X-flow), producing a cathode specific surface area of 93 m^2/m^3 (30). These membranes are expensive, however, and the properties of a UF membrane are not necessarily ideal for MFC applications. Inexpensive membranes that achieve low internal resistance in MFCs are therefore needed to further increase performance of tubular membrane cathodes.

Anion exchange (AEM) (31), cation exchange (CEM) (24, 26, 31), proton exchange (PEM) (32, 33), and bipolar (16) membranes have been previously used in MFCs, but only as a method to separate electrode chambers and decrease oxygen diffusion into the anode chamber. Kim et al. (31) showed that placing a CEM, AEM, or
Nafion™ membrane between the electrodes did not appreciably affect the internal resistance (84-88 Ω), based on a comparison to the same reactor without a membrane (84 Ω). The power generated using an AEM membrane (610 mW/m², 31), was slightly larger than obtained using a CEM (480 mW/m², 31) or Nafion (514 mW/m², 31) membrane due to charge transfer by negatively charged phosphate groups. Although AEM and CEM membranes have been tested as separators in MFCs, their performance as cathodes has not been previously explored. UF membranes have been used as both a separator and as the supporting material for a tubular cathode in MFCs (30, 31). However, in all cases these UF membranes have caused a large increase in internal resistance, and therefore have produced lower power densities than other materials (30, 31). These results demonstrate that the properties of ion exchange membranes are better suited for MFC applications than those of UF membranes.

In this study, we investigated the performance of two different ion exchange membranes when functioning as the cathodes in MFCs. By adding a graphite coating, and a non-precious metal catalyst (CoTMPP) to the surface of the flat membranes, we produced conductive cathodes capable of oxygen reduction at the surface. A flat membrane architecture was examined in order to permit power densities to easily be compared to previous studies, and therefore to eliminate any effects due to cathode geometry (i.e. the diameter or position of the tubes in the system). The performance of AEM (AMI-7001 without lamination, Membranes International Inc) and CEM (CMI-7000 without lamination, Membranes International Inc) membrane cathodes was compared to that achieved with a UF (UF-B0125, X-flow) membrane in electrochemical tests as well as fed-batch MFC experiments in terms of power production and CE.
Different catalyst locations (inside versus outside) and loadings, specific surface areas, and solution chemistry (solution conductivity) were examined in order to optimize performance.

6.2 Methods

6.2.1 Cathode Preparation

Membranes were cut into a circular shape to produce a projected surface area of 7 cm², and coated with graphite paint two times (ELC E34 Semi-Colloidal, Superior Graphite Co.) on one side of the membrane. A CoTMPP/carbon mixture (40% CoTMPP), prepared as previously described (34), was mixed with 5% Nafion solution and 100% isopropanol solution to form a paste (7 µl of Nafion + 7 µl of isopropanol per mg of CoTMPP/C catalyst) while stirring with glass beads. The paste was applied to the graphite painted side of the membrane at a loading of 0.5 mg/cm² CoTMPP (Co05) except as indicated. In some tests, the CoTMPP loading was varied over the range of 0.1 - 0.5 mg/cm² (0.1, Co01; 0.3, Co03; 0.5, Co05) by changing the mass of CoTMPP catalyst used in the paste. An AEM cathode with only graphite paint (C) and no CoTMPP paste was used as a non-catalyst control.

6.2.2 Electrochemical Cell Tests

The performance of UF, AEM, and CEM membrane cathodes with different catalysts applied to the air (outside, O) or liquid (inside, I) side of the membrane was
tested using chronopotentiometry in a two-chambered cubic electrochemical cell filled with a 50 mM PBS. Membranes are designated as: type-catalyst and loading-location as: UF-Co05-I, UF-Co05-O, AEM-Co05-I, AEM-Co05-O, CEM-Co05-I, and CEM-Co05-O. The cell was constructed from two plastic (Plexiglas) cylindrical chambers (2 cm long by 3 cm in diameter) each having a volume of 14 ml, and separated by a Nafion exchange membrane. For chronopotentiometry tests, the working electrode was the membrane cathode (7 cm² surface area) placed on one side of the cathode chamber and exposed to the air, the counter electrode was made of a platinum mesh (over 30 cm² surface area) located in the middle of the anode chamber, and the reference electrode was a Ag/AgCl electrode (RE-5B, Bioanalytical systems Inc) inserted into the middle of the cathode chamber with a 1-cm space between the reference and working electrodes. The catalyst-coated side of the membranes was tested either facing the solution (I) or facing the air (O). Chronopotentiometry studies using a potentiostat (PC4/750, Gamry Instruments Inc.) were conducted by applying a constant current (0 – 3000 mA/m²) for 30 min and recording the final value of the response potential (34). A curve of the cathode potentials against current densities was used to evaluate the performance of cathodes.

6.2.3 Membrane Cathode MFCs

All membrane cathode MFC tests were conducted using single-chamber cubic MFC reactors constructed as previously described (12). The anode electrode was an ammonia gas treated (35) graphite fiber brush (25 mm diameter × 25 mm length; fiber type: PANEX® 33 160K, ZOLTEK) with a surface area of 2235 cm² (95% porosity) (27).
The brush anode was positioned horizontally in the reactor with the metal end extending outside of the cell to provide a connection for the wire. The cathode was positioned against the end of the chamber with a 1-cm space between the tip of the brush anode and the membrane.

All brush anodes were inoculated using solution from an MFC (initially inoculated from the effluent of the primary clarifier of the local wastewater treatment plant) operated for over 1 year. Except as noted, all reactors were fed with a phosphate buffer nutrient solution containing acetate (0.8 g/L), phosphate buffer solution (PBS; 50 mM; Na₂HPO₄, 4.09 g/L and NaH₂PO₄·H₂O 2.93 g/L), NH₄Cl (0.31 g/L), KCl (0.13 g/L), trace mineral (12.5 mL/L), and vitamin solution (5 mL) having a solution conductivity of 7.5 mS/cm (12). Reactors were considered to be fully acclimated if the maximum voltage produced was repeatable for at least three batch cycles. To improve cathode performance, the AEM membrane was tested with a current collector consisting of a piece of stainless steel mesh (7 cm²; 30 mesh T316 stainless, 0.0065 wire diameter, TWP Inc.) placed against the coated AEM surface (AEM-Co05S-I). The effect of solution conductivity was examined using 200 mM PBS (20 mS/cm) in tests with the stainless steel mesh [AEM-Co05S-I (200)] as solution conductivity has been shown to increase power generation (8, 21). The medium in the reactor was refilled when the voltage dropped below ~ 10 mV. All experiments were performed at 30°C in a temperature-controlled room.

The internal resistance of AEM/CEM membrane cathode MFCs was measured in 50 or 200 mM PBS using electrochemical impedance spectroscopy (EIS) over a frequency range of 10⁵ to 0.005 Hz with a sinusoidal perturbation of 10 mV amplitude.
using a potentiostat (PC4/750, Gamry Instruments Inc.). The brush anode was used as the working electrode, and the membrane cathode was used as the counter and reference electrode as described previously (22).

6.2.4 Calculations and Measurements

The voltage ($V$) produced was measured using a data acquisition system (2700, Keithly, USA). Electrode potentials were measured using a multimeter (83 III, Fluke, UAS) and a reference electrode (Ag/AgCl; RE-5B, Bioanalytical systems Inc). Current ($I=V/R$), power ($P=IV$), and CE (based on the total input acetate) were calculated as previously described (8). Power and current density were either normalized to the projected area of membrane cathodes ($m^2$) or the total reactor volume ($m^3$). To obtain the polarization and power density curves as a function of current, external circuit resistances were varied from 40 - 2000 $\Omega$. For each test, one resistor was used for a full cycle (at least 24 hours), and for at least two cycles.

6.3 Results

6.3.1 Performance of Membrane Cathodes in Electrochemical Tests

The open circuit potentials (OCPs) of all membranes were similar (0.25 - 0.30 V), but their working potentials decreased to a range of -198 to -524 mV for current densities up to 3000 mA/m$^2$ (Figure 6.1). Current densities above 3000 mA/m$^2$ were higher than the working range of the membrane cathode MFCs and therefore were not examined in
electrochemical tests. The AEM membrane cathode with the catalyst facing the solution (AEM-Co05-I) produced the highest potentials, decreasing from 253 (OCP) to -198 mV (3000 mA/m²) (Figure 6.1). This membrane achieved potentials that were as much as 171 mV more positive than the next highest potentials using the CEM membrane cathode with the catalyst facing the water [254 (OCP) to -369 mV (3000 mA/m²)] (Figure 6.1). The UF membrane produced the most negative working potentials, with a voltage range of 245 to -493 mV with the catalyst facing the solution, and 290 to -524 mV with the catalyst exposed to air (0 - 3000 mA/m²) (Figure 6.1). Overall, it appears that membranes performed better when the catalyst faced the inside and directly contacted the liquid solution (Figure 6.1).

6.3.2 AEM and CEM Cathodes Tested in MFCs

Since AEM and CEM cathodes produced the highest potentials with the catalyst facing the solution, membranes with the catalyst oriented to the inside of the reactor (AEM-Co05-I and CEM-Co05-I) were further examined in single-chamber MFCs. Repeatable cycles of power production were rapidly achieved after only a few fed-batch cycles. Power density curves and polarization curves showed that the AEM cathode produced maximum power densities 57% greater than the CEM cathode, with a maximum power density of 449 ± 35 mW/m² (13.1 ± 1.0 W/m³, at 1603 mA/cm²) for the AEM cathode, and 286 ± 30 mW/m² (8.3 ± 0.9 W/m³, at 903 mA/cm²) for the CEM cathode (Figure 6.2A). The AEM cathode had a slightly lower internal resistance (47 ± 0 Ω) than the CEM cathode (55 ± 1 Ω).
The anode potentials were unaffected by the use of a CEM or AEM cathode (Figure 6.2B). The differences in power from these two MFCs were therefore a result of the differences in cathode potentials. The AEM cathode produced more positive potentials than the CEM cathode over the current density range of 0 – 1500 mA/m², in agreement with predictions based on chronopotentiometry (Figure 6.1, 6.2B). However, higher potential differences were observed for AEM and CEM cathodes in MFC systems than obtained in electrochemical tests. For example, at the same current density around 1500 mA/cm², the AEM and CEM cathode potential difference produced in MFC reactors was 177 mV (-132 mV for the AEM and -309 mV for the CEM cathode; Figure 6.2B), which is almost as double as that produced from electrochemical cells (100 mV potential difference, Figure 6.1).

The electron recovery efficiencies for both AEM and CEM membrane cathode MFCs were a function of current densities but were not appreciably different at the same current density (Figure 6.3). More than 50% of electrons were recovered from the substrate into electricity, with CE ranges of 56 to 66% for the CEM cathode MFC and 57 to 70% for the AEM cathode reactor over a current density from 500 to 2000 mA/m² (Figure 6.3).

6.3.3 The Effect of CoTMPP Loading on the AEM Cathode

The effect of catalyst loading was further investigated using AEM cathodes with the catalyst facing the solution. Power increased with the CoTMPP loading, with the maximum loading (AEM-Co05-I) producing 449 ± 35 mW/m² (13.1 ± 1.0 W/m³).
Decreasing the CoTMPP loading by 40% (AEM-Co03-I) reduced the maximum power by 28% to 321 ± 19 mW/m², and further decreasing the catalyst by 80% (AEM-Co01-I) decreased power by 56% to 198 ± 21 mW/m² (Figure 6.4A). In the absence of a catalyst, the AEM membrane cathode reactor (AEM-C-I) produced only 72 ± 10 mW/m² (2.1 ± 0.3 W/m³) (Figure 6.4A).

CEs measured at a fixed external resistance of 250 Ω ranged from 41 to 66% (Figure 6.5). The CEs increased with the amount of the CoTMPP catalyst as a result of the different times needed for a batch cycle. The highest catalyst loading produced a greater voltage and current density (Figure 6.4B), and thus a fed-batch cycle required the shortest operation time (Figure 6.5). Longer operation times were needed as the catalyst loading was decreased, allowing more oxygen to diffuse through the cathode into the liquid. This oxygen could be used by bacteria for aerobic degradation of the acetate, lowering the electron recovery and therefore decreasing the CE.

6.3.4 Improved Cathode Performance Using a Current Collector

The conductivity of the graphite coating on the AEM membrane is not as high as carbon electrodes, and thus reactor performance is reduced due to the resistance for electron transport across the electrode to the terminal. By using a stainless steel mesh (S) pressed against the coated side of the AEM cathode as a current collector, the internal resistance decreased from 47 ± 0 Ω (AEM-Co05-I) to 29 ± 0 Ω (AEM-Co05S-I). The maximum power produced increased by 28% to 574 ± 51 mW/m² (16.7 ± 1.5 W/m³,
Figure 6.6A), compared to the same system without the mesh (449 ±35 mW/m², 13.1 ± 1.0 W/m³).

Increasing the buffer concentration to 200 mM [AEM-Co05S-I(200)] further increased power output to 728 ± 3 mW/m² (21.2 ± 0.1 W/m³, Figure 6.6A). This is more than a 60% increase in power generation compared to the system lacking the current collector in 50 mM PBS. The internal resistance was reduced from 29 ± 0 Ω to 10 ± 1 Ω by using 200 mM PBS, as a result of the higher solution conductivity (from 7.5 to 20 mS/cm). The increase in power output was clearly due to the higher cathode potentials as the anode potentials were not affected by these changes in solution chemistry or by the use of the mesh (Figure 6.6B).

6.4 Discussion

Different types of ion exchange (AEM and CEM) and UF membranes can be used as MFC cathodes by coating them with an electrically conductive graphite paint and a non-precious metal catalyst (CoTMPP). The best performance was achieved using an AEM membrane with the catalyst coating directly in contact with the solution. A single-chambered brush anode MFC with this flat AEM cathode produced 449 ± 35 mW/m² (13.1 ± 1.0 W/m³) in a 50 mM PBS solution, and up to 728 ± 3 mW/m² (21.2 ± 0.1 W/m³) under optimal conditions (200 mM PBS, with a current collector). These power densities are comparable to 494 - 506 mW/m² (12.4 - 12.7 W/m³), produced using two carbon electrodes and a Pt cathode catalyst in the same single-chamber MFC (no membrane; 12, 20), and 610 mW/m² (15.3 W/m³; 31) in a two-chamber system with an AEM membrane.
placed in the middle of the reactor (all 4 cm electrode spacing). The CEs here (57 - 70\%) using the AEM membrane are much higher than those in the same reactor lacking a membrane (9 - 12\%, 12) or in the two-chamber system with an AEM separator (35 - 72\%; 31).

The reduced internal resistance and higher cathode performance achieved here using an AEM cathode, compared to a UF cathode, was based on comparisons using a flat-plate geometry for the cathode. Providing sufficient surface area with flat electrodes may be difficult for larger systems, and therefore we have previously suggested that tubular membranes could be used. The effect of a tubular geometry will need to be further investigated. In addition, the use of a current collector was also important for improving system performance. Adding a stainless steel mesh against the cathode substantially improved the cathode performance, primarily due to a 38\% reduction of the internal resistance. The electrical resistance of the coating on the AEM is 2 - 3 $\Omega$/cm, or about 10 times higher larger than that of the stainless steel mesh (0.1 - 0.3 $\Omega$/cm). The amount of stainless steel (or another non-corrosive and conductive material) needs to be reduced and optimized.

6.4.1 Economic Considerations

In addition to improvements in power densities and CEs, the continued development of low-cost materials is essential for scaling up MFCs and creating affordable treatment systems. The materials used here represent an order of magnitude decrease in cost compared to previously used materials on the basis of purchased prices,
but further reductions in costs are needed. The cost of the AEM membrane was $80/m², which is much less than the purchase price of either a UF membrane ($350/m²) or Nafion ($1400/m²). The catalyst was a non-precious metal, but its cost is high ($30/g). The amount of the catalyst is also an issue because the CoTMPP loading affected power production. In contrast, Pt content over a range of 0.1 - 2 mg/cm² does not appreciably affect power generation (34). The combined costs for an AEM cathode ($80/m²), graphite coating ($1/m²), and CoTMPP catalyst (0.5 mg/cm², $150/m²) is $231/m². This is only 14% of the cost for a carbon cathode ($1000/m²) with a Pt catalyst (0.5 mg/cm², $700/m²). These costs do not include binder or current collector costs, both of which would be needed for any system, or manufacturing costs.

The anodes will also need to be further optimized and reduced in cost. The price of graphite fibers on the basis of surface area ($0.58/m²) appears to be much less than that for carbon cloth ($1000/m²), but the cloth surface area is based on projected (geometric) surface area while the brush area is based on individual fibers. A 7 cm² carbon cloth anode costs $0.70, while graphite used in the brush anode costs less ($0.13; 2235 cm²). In addition, the surface area for the anode is likely far in excess of that needed for optimal power production, as the performance of the reactor is clearly constrained by the cathode performance as shown by the effect of the catalyst loading here and in other studies (23). Thus, anode costs could be substantially reduced by optimizing graphite fiber loading and other design aspects of the anode. The costs of both electrodes could probably be reduced by another order of magnitude with mass production and optimization of mass and types of construction materials.
6.4.2 Outlook

This procedure of converting non-conductive anion exchange membranes into electrically conductive and catalytically active cathodes provides a promising approach towards scale up of MFCs. When combined with graphite brush anodes, AEM cathodes produced a comparable power generation to a carbon electrode with the same surface area, but with much higher electron recovery efficiencies and reduced materials costs. Through the further development of tubular cathodes with lower internal resistances and greater electrical conductivities, this approach of using membrane cathodes appears very promising for scaling up MFCs with improved performance and reduced costs for wastewater treatment applications.

6.5 Acknowledgments

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6.6 Literature Cited


6.7 List of Figure and Table Captions

Figure 6.1: Cathode potentials as a function of current density measured in electrochemical cells for different membrane cathodes with different catalyst locations: ultrafiltration membrane (UF), anion exchange membrane (AEM), cation exchange membrane (CEM). Catalyst, CoTMPP with a loading of 0.5 mg/cm² (Co05), is located facing the solution (Inside, I) or the air (Outside, O).

Figure 6.2: (A) Power density (filled symbols), voltage (open symbols) and (B) electrode potentials (cathode open symbols, anode filled symbols) as a function of current density (projected to the cathode surface area) obtained by varying the external circuit resistance (40 - 2000Ω) for AEM and CEM cathode MFCs. (Error bars ±S.D. based on averages measured during stable power output in two or more separate batch experiments).

Figure 6.3: CEas as a function of current for AEM and CEM cathode MFCs. (Error bars ±S.D. based on averages measured during stable power output in two or more separate batch experiments).

Figure 6.4: (A) Power and (B) voltage as a function of the CoTMPP amount for the AEM cathode MFCs. CoTMPP with a loading of 0.5 (Co05), 0.3 (Co03), 0.1 (Co01), and 0/only graphite paint (C). (Error bars ±S.D. based on averages measured during stable power output in two or more separate batch experiments).
Figure 6.5: CEs as a function of operation times for the AEM cathode MFCs with different catalyst loadings (Fixed external resistance of 250 Ω. Error bars ±S.D. based on averages measured during stable power output in two or more separate batch experiments).

Figure 6.6: (A) Power density (filled symbols), voltage (open symbols) and (B) electrode potentials (cathode open symbols, anode filled symbols) as a function of current density obtained by varying the external circuit resistance (40 - 2000Ω) for AEM cathode MFCs using stainless steel mesh (S) and/or 200 mM PBS (200). (Error bars ±S.D. based on averages measured during stable power output in two or more separate batch experiments).
Figure 6.1: Cathode potentials as a function of current density measured in electrochemical cells for different membrane cathodes with different catalyst locations: ultrafiltration membrane (UF), anion exchange membrane (AEM), cation exchange membrane (CEM). Catalyst, CoTMPP with a loading of 0.5 mg/cm² (Co05), is located facing the solution (Inside, I) or the air (Outside, O).
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Figure 6.4: (A) Power and (B) voltage as a function of the CoTMPP amount for the AEM cathode MFCs. CoTMPP with a loading of 0.5 (Co05), 0.3 (Co03), 0.1 (Co01), and 0/only graphite paint (C). (Error bars ±S.D. based on averages measured during stable power output in two or more separate batch experiments).
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SELECTED PUBLICATIONS


