Although microbial fuel cells (MFCs) generate much lower power densities than hydrogen fuel cells, the characteristics of the cathode can also substantially affect electricity generation. Cathodes used for MFCs are often either Pt-coated carbon electrodes immersed in water that use dissolved oxygen as the electron acceptor or they are plain carbon electrodes in a ferricyanide solution. The characteristics and performance of these two cathodes were compared using a two-chambered MFC. Power generation using the Pt-carbon cathode and dissolved oxygen (saturated) reached a maximum of 0.097 mW within 120 h after inoculation (wastewater sludge and 20 mM acetate) when the cathode was equal size to the anode (2.5 × 4.5 cm). Once stable power was generated after replacing the MFC with fresh medium (no sludge), the Coulombic efficiency ranged from 63 to 78%. Power was proportional to the dissolved oxygen concentration in a manner consistent with Monod-type kinetics, with a half saturation constant of \( K_{SO} = 1.74 \text{ mg of O}_2/\text{L} \). Power increased by 24% when the cathode surface areas were increased from 22.5 to 67.5 cm² and decreased by 56% when the cathode surface area was reduced to 5.8 cm². Power was also substantially reduced (by 78% to 0.02 mW) if Pt was not used on the cathode. By using ferricyanide instead of dissolved oxygen, the maximum power increased by 50–80% versus that obtained with dissolved oxygen. This result was primarily due to increased mass transfer efficiencies and the larger cathode potential (332 mV) of ferricyanide than that obtained with dissolved oxygen (268 mV). A cathode potential of 804 mV (NHE basis) is theoretically possible using dissolved oxygen, indicating that further improvements in cathode performance with oxygen as the electron acceptor are possible that could lead to increased power densities in this type of MFC.

Introduction
A microbial fuel cell (MFC) uses bacteria to catalyze the conversion of organic matter into electricity (1–4). A MFC typically consists of two chambers, an anodic and an aerobic cathode chamber, separated by an ion conducting membrane. Bacteria at the anode oxidize organic matter and transfer electrons to the electrode that pass through an external circuit producing current. Protons migrate through the solution across the membrane to the cathode where they combine with oxygen and electrons to form water.

MFCs can be classified as two types depending on how electrons are transferred from bacteria to the electrode (anode): mediator MFCs, where electron shuttles or mediators are added into the system, and mediatorless MFCs, where no mediators need to be added (5–10). Mediators are generally toxic compounds (e.g., thionine, neutral red, and azure A); therefore, using them is impractical for economical electricity production (10, 11). On the other hand, mediatorless MFCs show significant potential for the harvesting of energy from waste organic material (10, 12, 13). In some cases, bacteria of the type present in mediatorless MFCs have electrochemically active redox enzymes on their outer membrane that can transfer electrons to external materials, and therefore, do not require exogenous chemicals to accomplish electron transfer to an electrode (14–16).

Research on mediatorless MFCs has so far concentrated primarily on two aspects of the system: on the bacteria able to produce electricity in the absence of a mediator and on the material used for the anode. It is known that several different bacteria can produce electricity in the absence of an exogenous mediator from chemicals such as glucose (Rhodoferax ferrireducens; ref 12), lactate, pyruvate, formate (S. putrefaciens; refs 3 and 17), benzoate (G. metallireducens; refs 4 and 10), acetate (G. sulfurreducens; ref 4), and hydrogen (G. sulfurreducens; ref 4 and 18). Even complex materials such as domestic wastewater can be degraded in MFCs (13), although bacteria that accomplish this have not yet been identified. Various materials have been used for the anode, including graphite rods (4, 12), carbon cloth (13), carbon paper (19), and woven graphite felt (9, 12).

Power densities in hydrogen fuel cells, which use Pt-coated electrodes, are orders-of-magnitude larger than those achieved so far in MFCs. Thus, it seemed likely to us that the performance of the MFC would be limited by the relatively low current generated at the anode by the bacteria and not by the performance of the cathode. Accordingly, most MFC studies report power generation normalized to the surface area of the anode. However, two studies that have reported very high power generation rates of 7200 mW/m² (20) and 3600 mW/m² (21) both used ferricyanide (potassium hexacyano-ferrate III) at the cathode. These power densities were larger than 0.3–787 mW/m² reported when dissolved oxygen was used (4, 10, 12, 13, 19, 22). The standard half-cell reactions and potentials for oxygen and ferricyanide at 25 °C (acidic solutions) are

\[
\begin{align*}
\text{O}_2 + 4\text{H}^+ + 4\text{e}^- &= 2\text{H}_2\text{O} & (E^\circ = 1.23 \text{ V}) \\
\text{Fe(CN)}_6^{3-} + \text{e}^- &= \text{Fe(CN)}_6^{4-} & (E^\circ = 0.36 \text{ V})
\end{align*}
\]

Ferricyanide in the cathode compartment is reduced to ferrocyanide; thus, the chemical must be replaced after it is depleted. However, oxygen can be constantly replenished by bubbling the water with air; thus, energy production from organic matter is sustainable in these systems.

While power generation has been greater in studies using ferricyanide, it is not known to what extent the cathode performance was responsible for this difference or if other factors, such as bacteria, substrate, or fuel cell construction were more important factors. The effect of the cathode on electricity generation has not previously been explored in two-chambered MFC studies. We therefore examined, using a two-chambered MFC operated under different conditions,
the effect of cathode performance on power generation. First, we compared power densities produced using the MFC with dissolved oxygen or ferricyanide. Next, we examined the effect of cathode size and dissolved oxygen concentration on performance for Pt-coated cathodes with dissolved oxygen as an electron acceptor. We found that cathode construction had a significant impact on power generation rates in MFCs despite their relatively lower power densities as compared to hydrogen fuel cells.

Materials and Methods

Culture and Medium. Dewatered sludge from an anaerobic digester was used as an inoculum in the anode compartment of the MFC. Anaerobic sludge, wastewater from a primary clarifier, and marine sediments have all been shown to be suitable biocatalysts for electricity production (13, 19, 23–25). Dewatered sludge (85% water) was collected from the Pennsylvania State University Wastewater Treatment Plant in State College, PA.

Acetate (20 mM) was used as an energy source in a nutrient solution (pH = 7.0) containing (per liter of deionized water): NaHCO3 (3.13 g/L), NH4Cl (0.31 g/L), Na2HPO4·H2O (0.75 g/L), KCl (0.13 g/L), Na2HPO4·2H2O (4.22 g/L), NaH2PO4 (2.75 g/L), and metal (12.5 mL) and vitamin (12.5 mL) solutions (26). In some experiments, oxygen was removed from the medium by sparging with nitrogen gas. All MFCs were operated at 30 °C in a constant temperature room.

MFC Construction and Operation. MFCs were constructed by joining two media bottles (310 mL capacity, Corning Inc., NY) with a glass tube containing a proton exchange membrane, or PEM (Nafion 117, DuPont Co., DE), clamped between the flattened ends of the glass tubes (inner diameter = 1.3 cm) fitted with two rubber gaskets (3.5 cm2 cross-section). The PEM was pretreated by boiling in H2O2 (30%) and deionized water, followed by 0.5 M H2SO4 and deionized water, each for 1 h, and then was stored in deionized water prior to being used. The anode used was made of carbon paper (2.5 × 4.5 cm). Unless stated otherwise, the cathode was the same size and was coated with a Pt catalyst (0.5 mg/cm2; 10% Pt) on one side (De Nora North America, Inc.). Electrodes were soaked in DI water for 1 day before tests. Copper wire inserted inside fluorinated ethylene propylene tubing (Chemfluor FEP Tubing; i.d.: 0.8 mm) was used to connect the circuit, and all exposed metal surfaces were sealed with a nonconductive epoxy (Dexter Corp. NJ).

The anode and cathode compartments were filled with medium (250 mL), and the anode chamber was filled with acetate (20 mM) and inoculated with sludge (2 g) in an anaerobic glovebox. The anode chamber was sealed with a rubber stopper and cap. The apparatus was removed from the glovebox, and current was calculated by measuring the voltage across a resistor (1000 Ω unless stated otherwise) using a multimeter. Both chambers were mixed using a magnetic stirrer. The cathode compartment was continuously sparged with air in experiments where oxygen was used as the oxidizer. Once the MFC demonstrated a repeatable cycle of power generation, the anode was removed and used in other systems containing different sized cathodes or ferricyanide (0.5–1.0 g of potassium ferricyanide) as the electron acceptor. In some tests, an Ag/AgCl reference electrode (0.195 V corrected to a normal hydrogen electrode; NHE) was placed into the anode or cathode compartments to determine individual electrode potentials.

Effect of Dissolved Oxygen Concentration. Two tests were used to determine the effect of dissolved oxygen (DO) on power generation: an unsteady-state test and a steady-state test. In the unsteady-state test, the cathode compartment was completely filled with air-saturated water (310 mL, no headspace) and sealed with a rubber stopper. A DO probe was inserted through the rubber septum of the cathode bottle, and both oxygen concentration and voltage were monitored. In the steady-state DO test, the cathode chamber was continuously sparged with a mixture of air and nitrogen gas, or pure oxygen, achieving a DO concentration in the range of 0–37.7 mg/L. Once a constant DO was achieved, voltages were measured every 30 min for 2–4 h and averaged.

Power generation was described as a function of DO concentration using an empirical Monod-type equation

\[
P = \frac{P_{\text{max}} \cdot \text{DO}}{K_{\text{DO}} + \text{DO}}
\]

where \(P_{\text{max}}\) is the maximum power, and \(K_{\text{DO}}\) is the half-saturation constant, indicating the concentration of DO that produces a power density one-half of the maximum value. Constants were determined using a nonlinear regression analysis (Curve fitter, SigmaPlot 2000).

Calculations. The system was monitored using a precision multimeter and a data acquisition system (Model 2700, Keithly Instruments, Inc.). Power (P) was calculated according to \(P = IV\), where \(I\) (C/s) is the current, and \(V\) (V) is the voltage. The Coulombic efficiency, \(E\) (%), was calculated as

\[
E = \left( \frac{C_{\text{ex}}}{C_{\text{th}}} \right) \times 100
\]

where \(C_{\text{ex}}\) is the total Coulombs calculated by integrating the current measured at each time interval (l) over time as \(C_{\text{ex}} = \sum I \cdot V(l)/R \cdot C_{\text{th}}\), the theoretical amount of Coulombs that is available from acetate oxidation, was calculated as \(C_{\text{th}} = \text{FbMV,}\) where \(F\) is Faraday’s constant (96 485 C/mol of e–), \(b\) is mol of electrons produced per mol of substrate (8 mol e–/mol of acetate), \(M\) is the acetate concentration (mol/L), \(V\) is the liquid volume (L), and \(r\) is the dilution rate.

Analytics. Dissolved oxygen was measured using a non-consumptive fiberoptic oxygen probe (FOXY-18G SF2000, Ocean Optics Inc., Dunedin, FL) and the manufacturer’s software (OOIFOXY oxygen sensor software, v. 1.67.15F). Prior to measuring samples, the probe was calibrated with oxygen-saturated medium (using air) in the same type of bottle (250 mL) and medium in which the DO was scavenged using sodium hydrosulphite.

Acetate was analyzed using a gas chromatograph (Agilent, 6890) equipped with a flame ionization detector and a 30 m × 0.32 mm × 0.5 µm DB-FFAP fused-silica capillary column. Samples were filtered through a 0.2 µm pore diameter membrane and were acidified using formic acid (0.65 M) before analysis. The temperature of the GC column was started at 60 °C, increased at 20 °C/min to 120 °C, and then at 30 °C/min to a final temperature of 240 °C for another 3 min. The temperatures of injector and detector were both 250 °C. Helium was used as the carrier gas at a constant pressure of 103 kPa.

The internal resistance of the system was evaluated from impedance data obtained using a Solartron 1287 electrochemical interface module (Solartron Analytical, Hampshire, England) connected to a Solartron 1255B frequency response analyzer and a personal computer and software (Zview Version 2.1b). Impedance data were obtained over a range of 100 Hz to 0.1 Hz, and the magnitude of the sinusoidal potential perturbation applied across the electrodes was ±10 mV. Z-plot and Z-view (Zview Version 2.1b) were used to control the impedance and to analyze the impedance data.

Results and Discussion

Reactor Acclimation and Stability of Power Generation. After a lag period of approximately 50 h after inoculation of the anode chamber, there was an exponential increase in cell voltage over the next ~50 h, reaching an initial maximum
voltage of 330 mV (Figure 1). An abiotic control did not generate any electricity (data not shown). When the acetate was completely consumed, the voltage decreased and the anode was transferred to a clean MFC (no wastewater inoculum or suspended bacteria). The medium was spiked several times over an extended period (~1000 h) to demonstrate a stable cycle of power generation, consisting of a rapid rise in power generation, followed by a gradual and then sharp decrease in voltage (Figure 2). Acetate was verified to be completely consumed following a rapid decrease in voltage. The electrode was transferred several times into fresh medium, and each time a stable cycle of voltage generation (320–340 mV) was produced without a lag phase (data not shown). This indicated that bacteria on the electrode were producing electricity and not suspended cells.

It was found over time that the maximum voltage achieved following a spike of acetate decreased (Figure 2). However, the Coulombic efficiency did not appreciably change, averaging 71 ± 7% (± SD; n = 4). Coulombic efficiencies obtained here were comparable to a range of 70–80% by Bennetto (27) using Escherichia coli and glucose and 81–83% by Chaudhuri and Lovley (12) using mixed cultures and glucose. These values are slightly larger than 50–65% reported by Thurston et al. (28) using Proteus vulgaris and glucose and much larger than those reported in some other studies (12%, ref 22; 3–12%, ref 13; and 0.04%, ref 29). Possible reasons for the lower Coulombic efficiencies in other studies include loss of substrate due to diffusion of oxygen through membrane, use of high circuit resistance, presence of other electron acceptors (such as \( \text{NO}_3^- \) or \( \text{SO}_4^{2-} \)), and differences in bacterial yield (19).

Attached biomass was observed on both sides of the PEM during the operation, but acetate was not detected in the cathode chamber. The growth of bacteria on the PEM of the cathode side suggested that acetate was diffusing into the cathode chamber from the anode chamber even though we could not measure an acetate diffusion coefficient in the apparatus using a new system. To determine if biomass on the PEM affected the power generation, the PEM was removed after 1000 h, and the biomass on the PEM was removed by rinsing with deionized water. When the cleaned PEM was placed back into the apparatus with fresh medium, the maximum voltage increased from 270 mV (before replacement) to 350 mV, indicating that bacterial growth on the PEM affected power generation.

**Effect of DO Concentration.** The effects of DO in the cathode compartment on power generation were evaluated under two different DO conditions: unsteady-state and steady-state. In unsteady-state tests, as soon as the cathode compartment was sealed, the DO concentration decreased steadily from 7.9 mg/L and was nearly completely consumed within 10 h. Power output decreased slowly over the first 2 h and then decreased much more rapidly for the next 8 h as the oxygen was depleted (Figure 3A). Power generation is shown as a function of DO in Figure 3B versus that obtained in the steady-state DO tests. Agreement between the two tests was not significantly different (T-test; \( p = 0.84 \)), although slightly less power is generally indicated in the steady-state tests.

Power output with water sparged with pure oxygen (38 mg/L DO) was 15.8% larger than that obtained using air-

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**FIGURE 1.** Current production over time in a microbial fuel cell inoculated with 2 g of dewatered anaerobic sludge (1000 \( \Omega \), 20 mM acetate).

**FIGURE 2.** Voltage generation over time in a MFC spiked with acetate at times shown by the arrows.

**FIGURE 3.** Effect of dissolved oxygen (DO) on power generation. (A) Power generated and DO concentration in an unsteady-state test. (B) Power generation as a function of DO in steady-state and unsteady-state tests. The regression line was fitted using all data shown.
saturated water (7.9 mg/L DO). The power was a maximum of 0.095 mW with air-saturated water versus 0.11 mW using oxygen-saturated water (Figure 3B). When power output was fitted using a Monod type of equation, a maximum power output was obtained of $P_{\text{max}} = 0.121$ mW and $K_{\text{D0}} = 1.82$ mg of O$_2$/L, using only the data for DO concentrations at or below air saturation produced. Using all data points produced values of $P_{\text{max}} = 0.118$ mW and $K_{\text{D0}} = 1.74$ mg of O$_2$/L (Figure 3B). The increase in the maximum power output using pure oxygen versus air is less than that expected based solely on energetics. Using the Nernst equation, the open circuit voltage would change in proportion to the oxygen partial pressure, according to $\Delta V \sim \ln(p_{\text{air}})$. For conditions examined here, this would predict a change in voltage by a factor of 1.56 or power by a factor of 2.44. Because power increased by only a factor of 1.16 here, other factors, such as the cathode overpotential, limited power output in tests with pure oxygen.

**Power Generation with Ferricyanide or Dissolved Oxygen.** As shown in Figure 4, power output was much greater using ferricyanide and an uncoated carbon electrode as a cathode as compared to that obtained using dissolved oxygen and the Pt-coated carbon electrode. Power generation was measured using a series of resistors (47–22,000 Ω) to determine the maximum power output as a function of current. The maximum power generated using ferricyanide was 0.17 mW (carbon electrode and ferricyanide) at currents of 0.4–0.6 mA, whereas the power reached a maximum of 0.097 mW at a current of 0.3 mA using the Pt-coated carbon electrode and dissolved oxygen. Thus, we see that the maximum power achieved with ferricyanide ion was 50–80% greater than that obtained with dissolved oxygen and the Pt cathode.

Using a plain carbon cathode (no Pt catalyst) decreased power output further, resulting in a maximum of only 0.02 mW. As large surface areas of the electrodes are needed to produce useful amounts of power in a MFC, the cost of Pt is an important component in the cost of the MFC. The substantially lower power densities produced in MFCs versus those in hydrogen fuel cells make it necessary to use Pt on the cathode.

The range of power densities for MFCs reported in the literature cannot be explained solely on the basis of the cathode solution. The maximum power densities reported in other studies using ferricyanide vary widely, with values of 7200 mW/m$^2$ (12.5 cm$^2$; ref 20), 4310 mW/m$^2$ (80 cm$^2$; ref 30), 3600 mW/m$^2$ (50.0 cm$^2$; ref 21), 32.3 mW/m$^2$ (65 cm$^2$ anode surface area; ref 12), and 3 mW (12 g of graphite felt; ref 9). These values are in some cases larger than those obtained in MFCs using dissolved oxygen, but they overlap in magnitude studies using oxygen. Power densities reported in studies using oxygen at the cathode were 788 mW/m$^2$ (80.0 cm$^2$ anode surface area; ref 23), 496 mW/m$^2$ (19), 33 mW/m$^2$ (12), 26 mW/m$^2$ (13), 20 mW/m$^2$ (22), 14.7 mW/m$^2$ (61.2 cm$^2$; ref 4), and 0.6 mW/m$^2$ (3). It is clear that differences in bacteria and MFC construction, which produce differences in MFCs that vary by orders-of-magnitude, are important factors in the maximum power density than the 50–80% difference produced by ferricyanide versus oxygen.

The increase in power when using ferricyanide versus oxygen is both a function of the different potentials of the chemicals as well as the mass transfer efficiency. We measured an open circuit potential (OCP) of 772 ± 17 mV (± SD; n = 3) using ferricyanide, 751 ± 30 mV using the Pt-coated electrode and oxygen, and 572 mV using the plain carbon electrode and oxygen. We also measured individual potentials based on the Ag/AgCl reference electrode and obtained a cathode potential of 332 mV and anode potential of −454 mV for ferricyanide and cathode potential of 268 mV and anode potential of −478 mV for oxygen (Pt cathode). The maximum potential that can be obtained using ferricyanide under these conditions is 360 mV, which is lower than cathode potential measured here (527 mV vs NHE). For the Pt-coated electrode, however, the cathode potential we obtained is substantially less than that calculated to be theoretically possible. The cathode potential E (NHE basis) for air and Pt at the cathode can be calculated under standard conditions using the Nernst equation (31).

$$E = 1.2288 + 0.0148 \log p_o - 0.05915 \text{pH} \quad (5)$$

Assuming an oxygen partial pressure of oxygen of $p = 0.2$, the maximum potential at a pH = 7 is 804 mV. This value is much larger than 268 mV (463 mV vs NHE) measured here, indicating that further improvements in cathode performance are possible using dissolved oxygen.

The use of Pt on the cathode was critical for achieving maximum power output with oxygen even though the power generation rates achieved with MFCs are low as compared to hydrogen fuel cells. When the Pt-coated carbon electrode was replaced with the plain carbon electrode, the cell voltage decreased from 302 to 136 mV. The cathode potential decreased from 108 to −203 mV, and the anode potential changed from −194 to −339 mV. This indicated that the reduced efficiency of the cathode lowered overall electricity generation, affecting the power generation at the anode as well. Thus, cathode catalysts remain essential to maximizing power output by MFCs.

**Effect of Cathode Surface Area on Power Generation.**

The voltages produced using the different cathode systems having different total surface areas are compared in Figure 5. The voltage for the ferricyanide cathode (22.5 cm$^2$) was 39% larger than that obtained with the Pt-coated carbon electrode with dissolved oxygen. The voltage produced by the Pt-coated carbon cathode and dissolved oxygen was reduced from 300 to 200 mV when the surface area was reduced from 22.5 to 5.8 cm$^2$. Increasing the surface area by 200% to 67.5 cm$^2$ increased the voltage by only 11%. Similar results were obtained using the uncoated carbon electrode, except the overall voltages were much lower than those with the Pt-coated electrode. These results demonstrating that the surface area of the cathode limited power generation are different than those found by Dentel et al. (24) using a sediment MFC having graphite foil electrodes (no Pt) to treat wastewater sludges. They found that the cathode could be up to five times smaller than the anode to generate optimum power (0.02 mW, 200 cm$^2$ anode).

When ferricyanide was used in the cathode, the voltage was maintained at 400–420 mV regardless of the presence or absence of Pt for surface areas ranging from 5.8 to 67.5 cm$^2$ (0.25–3.0 times the anode surface area). There was also a slight drop in voltage measured using the 5.8 cm$^2$ cathode,
but this change was less than that observed using the dissolved oxygen electrodes. The much larger working voltages and power levels achieved with the ferrocyanide solution versus that obtained with dissolved oxygen (Figures 4 and 5) is most directly a function of the greater working potentials of the cathode. Other differences in the system, for example, their internal resistances, were relatively small. The internal resistance with the 22.5 cm² Pt cathode and dissolved oxygen was 960 Ω, while the internal resistance of the system with ferrocyanide was only 17% less (800 Ω). In addition, changing the size the ferrocyanide cathode from 22.5 to 2 cm² (a factor of 11.3) increased the internal resistance by less than 2.2%. Thus, the differences in the power densities achieved with the two different types of cathodes reflect the greater mass transfer efficiency with concentrated ferrocyanide than that obtained with dissolved oxygen.

It appears that the working cathode potentials using dissolved oxygen can be improved as values obtained here are still below those theoretically possible to achieve using Pt-coated carbon electrodes. In addition, we have only considered here the use of an aqueous-phase cathode, or a two-chambered system where the cathode is immersed in water. It has also been shown that MFCs can be designed as a single chamber so that the cathode is directly exposed to air, making it unnecessary to use an energy-intensive process such as air-sparging to transfer oxygen to the cathode (13, 19). While two-chambered systems based on aqueous phase cathodes will continue to be used in the laboratory, it is likely that practical applications of MFCs will use more efficient designs where the cathode is directly exposed to air. Thus, the type and size of the cathodes used in different MFCs should be considered when noting the power densities achieved in these systems.

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Note Added after ASAP Posting

This paper was released ASAP on 08/06/2004 with an error in the fourth paragraph of the Materials and Methods section and in the symbols of Figure 3B. The correct version was posted on 08/19/2004.