Production of Electricity from Acetate or Butyrate Using a Single-Chamber Microbial Fuel Cell

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Hydrogen can be recovered by fermentation of organic material rich in carbohydrates, but much of the organic matter remains in the form of acetate and butyrate. An alternative to methane production from this organic matter is the direct generation of electricity in a microbial fuel cell (MFC). Electricity generation using a single-chambered MFC was examined using acetate or butyrate. Power generated with acetate (800 mg/L) (506 mW/m² or 12.7 mW/L) was up to 66% higher than that fed with butyrate (1000 mg/L) (305 mW/m² or 7.6 mW/L), demonstrating that acetate is a preferred aqueous substrate for electricity generation in MFCs. Power output as a function of substrate concentration was well described by saturation kinetics, although maximum power densities varied with the circuit load. Maximum power densities and half-saturation constants were \( P_{\text{max}} \) = 661 mW/m² and \( K_s \) = 141 mg/L for acetate (218 Õ) and \( P_{\text{max}} \) = 349 mW/m² and \( K_s \) = 93 mg/L for butyrate (1000 Õ). Similar open circuit potentials were obtained using similar acetate and butyrate (795 mV). Current densities measured for stable power output were higher for acetate (2.2 A/m²) than those measured in MFCs using butyrate (0.77 A/m²). Cyclic voltammograms suggested that the mechanism of power production in these batch tests was by direct transfer of electrons to the electrode by bacteria growing on the electrode and not by bacteria-produced mediators. Coulombic efficiencies and overall energy recovery were 10–31 and 3–7% for acetate and 8–15 and 2–5% for butyrate, indicating substantial electron and energy losses to processes other than electricity generation. These results demonstrate that electricity generation is possible from soluble fermentation end products such as acetate and butyrate, but energy recoveries should be increased to improve the overall process performance.

Introduction

Harvesting products from wastewater in order to make the process more economical and sustainable is the next frontier in wastewater treatment (1, 2). Hydrogen production from wastewater by biological fermentation has drawn much attention as a method of producing a valuable product during treatment of wastewaters containing high concentrations of carbohydrates (3–7). One mole of glucose can theoretically be converted into 12 mol of hydrogen, but the maximum yield via known fermentation routes is only 4 mol of hydrogen when acetate is the sole byproduct. While the maximum efficiency of hydrogen production is therefore 33%, typically only 15% of the energy is recovered as hydrogen (2, 8) with the remainder of the organic matter present as fatty acids and alcohols.

To improve the economics of hydrogen production from wastewater, additional processes are needed to recovery the remaining energy. One approach is to link hydrogen production with methane production by using a two-stage process (2). Although two-stage anaerobic treatments have been used to make methane, it has not yet been proven outside of the laboratory that hydrogen can be recovered at high concentrations from the first stage using actual wastewaters. A second approach is to use phototrophic bacteria to recover additional hydrogen from the byproducts of hydrogen fermentation (9, 10). Although solar energy is free, the availability of sufficient land area and the instability of sufficient solar energy at the plant would make such a process difficult for wastewater treatment applications. A third approach is to recover the remaining energy directly as electricity in a microbial fuel cell (MFC). While electricity production has been shown in MFCs using glucose or acetate, much remains to be done in order to use this technology for wastewater treatment.

Bacteria present in wastewater, anaerobic reactor sludges, and marine sediments have been shown to produce electricity in a MFC (11–14). Bacteria that have been identified to be capable of making electricity in fuel cells, most of which are metal-reducing bacteria, include \textit{Geobacter sulfurreducens} (15, 16), \textit{Geobacter metallireducens} (13, 16), \textit{Shewanella putrefaciens} (17, 18), \textit{Clostridium butyricum} (19), \textit{Rhodoferax ferrireducens} (20), and \textit{Aeromonas hydrophila} (15). It has also been recently shown that electricity generation in an MFC resulted in large part from the production of mediators, or electron shuttles, by a microbial community consisting of primarily three bacteria: \textit{Alcaligenes faecalis}, \textit{Enterococcus faecium}, and \textit{Pseudomonas aeruginosa} (12).

Many MFCs contain two chambers (16–18, 20). One chamber contains electrochemically active bacteria growing under anaerobic conditions that grow as a biofilm attached to the anode. The other chamber is kept aerobic by sparging water with air and contains the cathode. The two chambers are typically separated by a proton exchange membrane (PEM), which allows the transfer of protons from the anode to cathode chamber and that helps to physically block oxygen diffusion into the anode chamber. Recently, single-chamber MFCs have been developed that use a cathode exposed directly to air instead of air-sparged water (11, 21, 22). There are several advantages of using a single-chamber MFC versus a two-chambered system: increased mass transfer to the cathode; decreased operating costs, because it is not necessary to sparge the water; an overall decrease in reactor volume; and a simplified design. Power output can further be increased in a single-chamber MFC by removing the PEM (11). Although there is increased oxygen diffusion into the anode chamber in the absence of the PEM, the formation of an aerobic biofilm on the cathode inner surface (facing the anode) removes any oxygen that diffuses into the chamber, preventing the loss of anaerobic conditions in the anode chamber. The lack of a PEM also substantially decreases the cost of the materials needed to make a MFC.

The primary fermentation end products during biogas production are acetate and butyric acids. Thus, to link...
a MFC to biohydrogen production it must be shown that power can be generated from degradation of these compounds. However, there are no previous studies on electricity production from butyrate, which can account for up to 70% of the aqueous byproducts of hydrogen production from sugar fermentation (8). Power generation from acetate in two-chambered MFCs is well known, but there have been no previous reports of power generation using acetate in single-chambered systems. Here we demonstrate that electricity can be generated from butyrate in a single-chambered MFC, and we compare power densities obtained from acetate and butyrate with those previously obtained in the same system using glucose (11).

**Methods**

**MFC Construction and Operation.** The membrane-free single-chamber MFCs consisted of an anode and cathode placed on opposite sides in a plastic (Plexiglas) cylindrical chamber 4 cm long by 3 cm in diameter (empty bed volume of 28 mL) as previously reported (11). The anode electrode was made of toray carbon paper (without wet proofing; E-Tek). The cathode was carbon paper containing 0.35 mg/cm² platinum (E-Tek). Platinum wire was used to connect the circuit.

Following inoculation and stable power generation using domestic wastewater (after four transfers of wastewater into the reactor, 140 h), a nutrient medium containing acetate or butyrate was added to the anode chamber. Acetate (80–800 mg/L) and butyrate (75–1000 mg/L) concentrations were varied to determine power output as a function of substrate concentration. No precautions were taken to remove dissolved oxygen from the medium or to maintain anaerobic conditions in the anode chamber. Experiments were conducted at least in duplicate, in a constant-temperature room (30 °C).

**Calculations.** Voltage (V) was measured using a multimeter with a data acquisition system (2700, Keithley) and used to calculate the power (P) according to $P = IV$. Power was normalized by either the cross-sectional area of the anode, $A$, or by the liquid volume, $V$. The Coulombic efficiency was calculated as, $E = C_i/C_i^* \times 100\%$, where $C_i$ is the total coulombs calculated by integrating the current over time. $C_i$ is the theoretical amount of coulombs that can be produced from either sodium acetate ($i = a$) or sodium butyrate ($i = b$), calculated as

$$C_i = Fb_S N_i / M_i \quad (1)$$

where $F$ is Faraday’s constant (96 485 C/mol-electrons), $b_i$ the number of moles of electrons produced per mole of substrate ($b_a = 8$, $b_b = 20$), $S_i$ the substrate concentration, and $M_i$ the molecular weight of the substrate ($M_a = 82$, $M_b = 110$). Overall energy recovery was calculated as, $E_{\text{recovery}} = E_{\text{recovery}} / C_i^* \times 100\%$, where $E_{\text{recovery}}$ is the total energy calculated by integrating the power over time. $E_{\text{recovery}}$ is the theoretical amount of energy that can be produced from the substrate, calculated as

$$E_{\text{recovery}} = \Delta HS_{\text{recovery}} / M_i \quad (2)$$

where $\Delta H$ is the enthalpy change of the following reaction under standard conditions:

$$C_2H_4O_2 + 2O_2 \rightarrow 2CO_2 (g) + 2H_2O (l) \quad (3)$$

$$C_4H_9O_2 + 5O_2 \rightarrow 4CO_2 (g) + 4H_2O (l) \quad (4)$$

Power was modeled as a function of substrate concentration ($S$) using an empirical Monod-type equation as

$$P = \frac{P_{max} S}{K_s + S} \quad (5)$$

**FIGURE 1.** Voltages generated using acetate at different concentrations.

$P_{max}$, the maximum power and $K_s$ the half-saturation constant were determined using the Solver function in Microsoft Excel 2002.

**Analysis.** Acetate and butyrate concentrations were 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 followed the same procedure described previously (11). Electrode open circuit potentials (OCP) and working potentials were measured using a multimeter (83 III, Fluke) with Ag/AgCl reference electrode (RE-5B, Bioanalytical Systems).

**Cyclic Voltammetry (CV).** Cyclic voltammetry (PC4/750, potentiostat, Gamry) was used to characterize the oxidation-reduction reactions on the electrode surface by measuring the current response at an electrode surface to a specific range of potentials in an unstirred solution at a scan rate of 20 mV/s (minimum of 5 scans). The anode was the working electrode, and the counter electrode was the MFC cathode with a Ag/AgCl reference electrode. The potentials were originally in the range of −800 to 200 mV, but since peaks were only found in the range of −500 to 0 mV, this smaller range was used in the latter experiments.

**Results**

**Power Generation as a Function of Substrate Concentration.** Following inoculation and stable power generation of the reactor with wastewater, a stable voltage was generated after three additional transfers (~60 h) using a medium containing acetate (80 mg/L) or butyrate (75 mg/L) into the anode chamber. An example of the cycle of power generation for reactors fed different initial acetate concentrations (80–800 mg/L) is shown in Figure 1. A plot of the maximum power output at each initial substrate concentration demonstrated saturation kinetics at three different circuit loads of 218, 1000, and 5000 Ω (Figure 2A). A maximum power density of $P_{max} = 661$ mW/m² and half-saturation constant of $K_s = 141$ mg/L ($R^2 = 0.997$) was obtained using a 218 Ω resistor, while those using 1000 and 5000 Ω resistors were $P_{max} = 343$ mW/m² and $K_s = 43$ mg/L ($R^2 = 0.999$) and $P_{max} = 86$ mW/m² and $K_s = 9$ mg/L ($R^2 = 0.999$), respectively. For the butyrate-fed MFC and a 1000 Ω resistor, the maximum power was approximately the same with $P_{max} = 349$ mW/m² and a half-saturation constant of $K_s = 93$ mg/L ($R^2 = 0.887$) (Figure 2B).

**Power as a Function of Current Density.** By varying the circuit resistance from 70 to 5000 Ω (current densities of 0.2–2.2 A/m²) with acetate (800 mg/L) as the substrate, a maximum power of 506 mW/m² (12.7 mW/L) was obtained at a current density of 1.8 A/m² (218Ω) (Figure 3). The MFC using butyrate (1000 mg/L) as substrate generated a maximum power of 305 mW/m² (7.6 mW/L) at a current density of 0.65 A/m² (1000 Ω).

**Electrode Potential.** Electrode open circuit potentials and working potentials were measured for each substrate by varying the circuit load. With acetate (800 mg/L) as the substrate, the anode and cathode open circuit potentials were −480 ± 15 and 318 ± 10 mV (Ag/AgCl reference electrode),
respectively, with an OCP of 798 mV (Figure 4). Similar results were obtained using butyrate (800 mg/L), with an anode potential of -475 (15 mV, a cathode potential of 319 (10 mV, and an OCP of 794 mV. The cathode exhibited an overpotential for both acetate and butyrate, with the voltage decreasing sharply from the open circuit potential to 105 mV for acetate and 130 mV for butyrate. For both substrates, the anode working potential increased slightly with current density at the lower current density (0-1.8 A/m² for acetate and 0-0.66 A/m² for butyrate), but it became unstable at a higher current (over 2.2 A/m² for acetate and 0.77 A/m² for butyrate) (Figure 4). At the lower current density, current densities were limited by high resistance. At a lower circuit resistance (<218 ¿ for acetate and <1000 ¿ for butyrate), the bacteria growing on the anode were unable to transfer electrons fast enough into the circuit causing an increased overpotential. Acetate sustained a higher current density that was 3 times that measured for butyrate (2.2 versus 0.77 A/m²), indicating faster bacterial uptake of acetate than butyrate.

Substrate Degradation and Coulombic Recovery. Substrate removal was nearly complete (>99% removal for acetate, ~98% for butyrate) when the voltage of the batch experiments (1000 ¿) was reduced to <0.030 V for all tests at different initial substrate concentrations. The overall Coulombic efficiency was a function of substrate concentration and circuit resistance. Coulombic efficiency decreased from 28.3 to 13.2% when the acetate concentration increased from 80 to 800 mg/L (1000 ¿) (Figure 5). At a fixed initial acetate concentration (800 mg/L), decreasing the circuit resistance from 5000 to 70 ¿ increased Coulombic efficiency from 9.9 to 31.4% (Figure 6). The Coulombic efficiencies with butyrate were lower than those of acetate, but decreased (15-7.8%) with increased concentrations of butyrate (75-1000 mg/L). In tests with butyrate, low concentrations of acetate (<50 mg/L) were detected in solution, indicating that some butyrate was degraded into acetate. Electricity could have been generated from butyrate degradation to acetate according to

\[ C_4H_8O_2 + 2H_2O \rightarrow 4C_2H_4O_2 + 4H^+ + 4e^- \]  

Alternatively, it is possible that butyrate was first converted into acetate by butyrate-degrading acetogenic bacteria (23).

Energy Recovery. While Coulombic efficiency indicates recovery of electrons, the overall energy recovery of the system represents the energy harvested as electricity from bacteria versus that lost to other processes. When acetate was used, 6.5-5.1% of the energy was recovered at initial acetate concentrations of 80-800 mg/L. Energy recovery was increased from 3.0 to 7.2% by increasing circuit resistance (from 70 to 218 ¿) for lower circuit loads. Increasing the resistance further to 5000 ¿ in acetate-fed cells decreased the overall energy recovery to 4.7%. In butyrate-fed MFC tests, energy recovery ranged from 2 to 5%.

Cyclic Voltammograms. Extracellular electron transfer in mediator-less MFCs can occur by two mechanisms: by
membrane-bound proteins, mediators, or electron shuttles, produced by the bacteria and excreted into the environment (12, 24). To examine whether electron shuttles were generated and contributed to the electricity generation in this system, CV was performed using three samples: anodes obtained from a MFC during stable power generation; anodes at the end of a cycle of electricity generation (when the substrate was consumed); and a new anode (no biofilm) present in the same medium used in other tests.

Using anodes from active MFCs, oxidation peaks in the forward scans of the voltammograms were observed at −280 mV (vs Ag/AgCl) (1100 µA) for the acetate-fed MFC and −300 mV (343 µA) for the butyrate-fed MFC (Figure 7). During the reverse scan, additional oxidation peaks were found at −340 mV (vs Ag/AgCl) (608 µA) for acetate and −370 mV (vs Ag/AgCl) (190 µA) for butyrate. No reduction peaks were found in reverse scans. However, two redox couples were observed in voltammograms (−304 and −377 mV) using anodes obtained at the end of the batch electricity generation cycle (2 mV, 1000 Ω) (Figure 8A). This could be evidence of mediator production by the mixed culture. However, based on the low current of 50–150 µA, the concentration of mediators would be quite low. These mediators, if present, were held in the biofilm. When a voltammogram was obtained using the same solution, but with a new anode (no biofilm), no redox couples were detected (Figure 8B). These results make it appear likely that the main mechanism of power production in these batch tests was by direct transfer of electrons to the electrode by bacteria containing enzymes directly attached to their cell membranes.

Discussion

The electricity generation from either acetate or butyrate using a single-chamber MFC is a proof-of-concept demonstration of a technology to link MFCs with biohydrogen production by fermentation. Biochemical routes that lead to acetate produce more hydrogen than those that lead to butyrate production. It was shown here that the power generated from MFCs fed acetate (506 mW/m² or 12.7 mW/L) was up to 66% higher than those fed with butyrate (305 mW/m² or 7.6 mW/L). The predominant oxidation peak intensity of CV also reflected the electron-transfer rate difference from acetate and butyrate to electrodes with the maximum current reached 1100 µA for the acetate-fed anode but only 343 µA for the butyrate-fed one. Taken together, these results demonstrate that acetate is a preferred aqueous substrate for both hydrogen production and electricity generation in MFCs.

The power generated here by using a direct-air cathode MFC without a PEM was over 54% (acetate) and 57% (butyrate) higher than power levels obtained using a MFC in the presence of the PEM (328 mW/m² with acetate: 194 mW/m² with butyrate). This greater level of power generation in the absence of a PEM was previously reported in tests using glucose or domestic wastewater as substrates (11). By removing the PEM in those studies, power output was 5.2 (wastewater) and 1.9 times greater (glucose) than power levels obtained in MFCs containing a PEM. Since PEMs such as Nafion are quite expensive, the removal of PEM greatly decreases the cost for MFC construction and thus further increases the possibility of economical power generation in MFCs linked with hydrogen production.

Further Improvements Needed in MFC Performance.

One aspect that needs to be improved in MFC performance is power density. Based on available anode surface area and maximum bacterial growth rates, the maximum power that can be produced in a mediator-less MFC was estimated on the order of 10³ mW/m² by assuming a monolayer of bacteria on an electrode surface (11). However, the presence of additional bacteria in a biofilm capable of producing mediators could greatly increase power. The potential of large increases in power production using bacteria that produce their own mediators was demonstrated by Rabaey et al. (12). They obtained a power density of 4310 mW/m² using a mixed culture primarily consisting of A. faecalis, E. faecium, and P. aeruginosa. The use of cyclic voltammograms in their study demonstrated that power production occurred primarily as a result of mediators, in contrast to our study which shows that mediators were largely absent. Long-term enrichment and cultivation of bacteria in MFCs could lead to increased power production if mediators remain in the system. In our tests, we found some evidence of mediator production by the biofilm but did not observe mediators in solution. Thus, the contribution of exogenous mediators to MFCs, particu-
larly in continuous-flow systems where they could diffuse out of the system, is unknown.

The other aspect of MFC operation that needs to be improved is Coulombic efficiency and overall energy recovery. The Coulombic efficiency of the air–cathode MFC without a PEM used in this study was 10–30%. This was greater than 0.04% reported for starch processing wastewater (19) but comparable to 3–12% found for domestic wastewater (22). However, these values are substantially lower than 89% reported by Rabaey et al. (25) using glycol as substrate. In their system using an enriched culture, potassium hexacyano-

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There are several factors that could be responsible for low electron and energy recoveries in MFCs used here. First, removal of the PEM increases oxygen transfer into the anode chamber. Oxygen diffusion through the cathode could account for 21–50% of acetate loss based on a previously measured oxygen-transfer rate of 0.187 mg/h (11). Second, substrate loss is also possible due to methanogenesis. The high concentrations of acetate and anaerobic conditions favor methane production in the anode chamber. Third, substrate is used for bacterial growth and production of biomass. It may be that the bacteria grown in our MFC tests have higher biomass yields than other bacteria such as Geobacter sp. used in pure culture studies (16). Fourth, alternate electron acceptors, such as sulfate present in the medium, can also reduce electron recovery. Energy recovery relies on all the same factors as electron recovery, but additionally depends on the energy used by the bacteria versus that available to drive electron flow. The sooner electrons are transferred from enzymes in the bacterial respiratory pathway (i.e., at the level of a quinone versus that of a cytochrome), the greater the potential and the larger the energy recovery.

To increase electron and energy recovery, oxygen diffusion must be reduced from the cathode into the anode chamber. This could be achieved by further increases in the cathode efficiency making it possible to use smaller cathodes. Alternatively, coatings could be placed on the cathode that restrict oxygen diffusion by allow for proton transfer to the cathode. It may be possible to limit methanogenesis by controlling pH or through treatment of the inoculum to reduce the potential for methanogen growth. Further advances in the design and operation of MFC are needed in order to accomplish greater overall MFC performance.

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