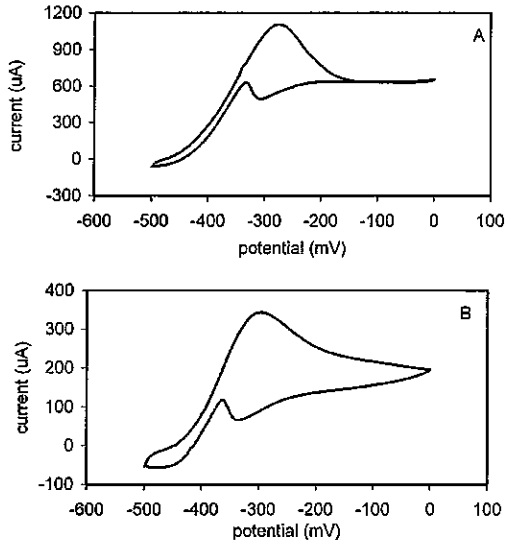

FIGURE 7. Cyclic voltammograms of the anode of an (A) acetate-fed and (B) butyrate-fed MFC (the anode chamber solution contained medium and the substrate).

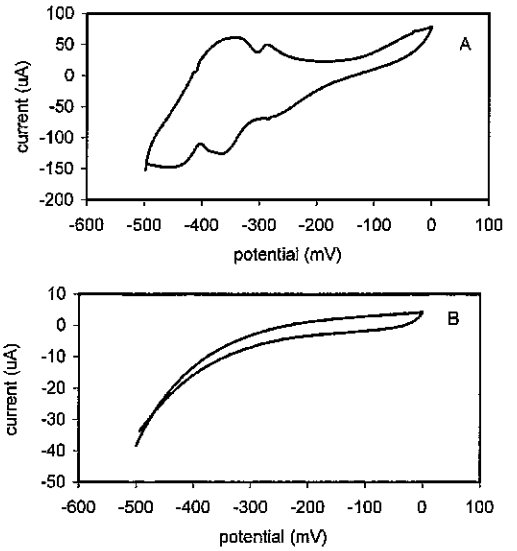

FIGURE 8. Cyclic voltammograms of (A) the anode with a biofilm and (B) a new anode (no biofilm). (Both tests were conducted with the anodes placed in the solution obtained from the anode chamber of an acetate-fed MFC at the end of a batch test).

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/m^2 or 12.7 mW/L) was up to 66% higher than those fed with butyrate (305 mW/m^2 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/m^2 with acetate; 194 mW/m^2 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 10^3 mW/m^2 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/m^2 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 glucose as substrate. In their system using an enriched culture, potassium hexacyanoferrate was used as oxidant instead of oxygen, and a PEM was used to separate the anode and cathode chambers. Energy recovery in their system was 65% versus only 2−7% obtained here with a nonenriched inoculum.

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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