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Performance and Bacterial Consortium of Microbial Fuel Cell Fed with Formate†

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Microbial fuel cells (MFCs) have been used to enrich microbes oxidizing formate with concomitant electricity generation. Medium containing formate was fed continuously to MFCs. MFCs showed approximately 1 mA of current after 4 months of operation. Over 90% of formate supplied was removed in MFCs, while Coulombic efficiency was only 5.3% indicating substantial electron and energy losses rather than electricity generation. Denaturing gradient gel electrophoresis (DGGE) showed that a formate-utilizing acetogenic bacterium (*Acetobacterium* sp.), an acetate-oxidizing metal reducer (*Geobacter* sp.), and another formate utilizer (*Arcobacter* sp.) were mainly detected on the electrode. This result indicates that some formate was consumed by acetogenic bacteria to make acetate, and acetate was used by acetate-utilizing electrochemically active bacteria (EAB) (e.g., *Geobacter* sp.). Additionally, formate was oxidized by nonelectrochemically active bacteria under microaerobic conditions in the anode compartment of the MFCs.

1. Introduction

A mediator-less microbial fuel cell (MFC) is a device that converts chemical energy into electrical energy with the aid of catalytic reactions of electrochemically active bacteria (EAB).[1,2] Chang et al.[3] defined EAB as bacteria that possess the ability to transfer electrons from oxidized fuel (substrate) to a working electrode without mediators, making it possible to establish mediator-less MFCs. Dissimilatory metal-reducing bacteria (DMRB), which are capable of the reduction of solid metal oxides, are known EAB species, including *Geobacter* and *Shewanella* spp.[2,4,5] It was shown that the anode electrode in MFCs served as the electron acceptor for growth and metabolism of EAB, which are capable of current production in the absence of a mediator.[5]

Not only did single strains demonstrated this ability in MFC,[6–8] but some bacterial consortia also have been enriched on anodes of MFCs, using organic materials in wastewater as fuel and recovering electrons as the current.[9–13] This enrichment technique was explored using sludge collected from a corn-processing wastewater treatment plant as the inoculum. During the operation, repeated wastewater replacements were coupled with current increase.[11] Furthermore, enrichments of MFCs were made with continuous feeding of various different fuels, including artificial wastewater containing acetate,[12] propionate, or artificial wastewater containing glucose and glutamate.[9] In most cases, current from MFCs was stably generated within 3 weeks under given feeding rates and fuel concentrations. The results revealed that the bacterial population in the MFC was different from that in the original inoculum[11–13] and microbial populations in MFCs were dependent on the fuel used.

Among the previous research on MFCs, nonfermentative fatty acids such as acetate and butyrate were favorably used as the fuels[9,10,12,14] to enrich EABs, which are capable of current production without co-metabolism of fermentation. The MFCs fed fermentable substrates such as glucose and glutamate[9] showed more diverse bacterial populations on anode electrodes than those fed nonfermentable substrates such as acetate.[12]

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(1) Allen, R. M.; Bennetto, H. P. *Appl. Biochem. Biotechnol.* **1993**, *39/40*, 27–40.

(2) Kim, B. H.; Kim, H. J.; Hyun, M. S.; Park, D. H. *J. Microbiol. Biotechnol.* **1999**, *9*, 127–131.

(3) Chang, I. S.; Moon, H.; Bretschger, O.; Jang, J. K.; Park, H. I.; Nealson, K. H.; Kim, B. H. *J. Microbiol. Biotechnol.* **2006**, *16*, 163–177.

(4) Methé, B. A.; Nelson, K. E.; Eisen, J. A.; Paulsen, I. T.; Nelson, W.; Heidelberg, J. F.; Wu, D.; Wu, M.; Ward, N.; Beanan, M. J.; Dodson, R. J.; Madupu, R.; Brinkac, L. M.; Daugherty, S. C.; DeBoy, R. T.; Durkin, A. S.; Gwinn, M.; Kolonay, J. F.; Sullivan, S. A.; Haft, D. H.; Selengut, J.; Davidsen, T. M.; Zafar, N.; White, O.; Tran, B.; Romero, C.; Forberger, H. A.; Weidman, J.; Khouri, H.; Feldblyum, T. V.; Utterback, T. R.; Van Aken, S. E.; Lovley, D. R.; Fraser, C. M. *Science* **2003**, *302*, 1967–1969.

(5) Kim, H. J.; Hyun, M. S.; Chang, I. S.; Kim, B. H. *J. Microbiol. Biotechnol.* **1999**, *9*, 365–367.

(6) Bond, D. R.; Lovley, D. R. *Appl. Environ. Microbiol.* **2003**, *69*, 1548–1555.

(7) Chaudhuri, S. K.; Lovley, D. R. *Nat. Biotechnol.* **2003**, *21*, 1229–1232.

(8) Ringeisen, B. R.; Henderson, E.; Wu, P. K.; Pietron, J.; Ray, R.; Little, B.; Biffinger, J. C.; Jones-Meehan, J. M. *Environ. Sci. Technol.* **2006**, *40*, 2629–2634.

(9) Choo, Y. F.; Lee, J.; Chang, I. S.; Kim, B. H. *J. Microbiol. Biotechnol.* **2006**, *16*, 1481–1484.

(10) Jong, B. C.; Kim, B. H.; Chang, I. S.; Pauline, W. Y. L.; Choo, Y. F.; Kang, G. S. *Environ. Sci. Technol.* **2006**, *40*, 6449–6454.

(11) Kim, B. H.; Park, H. S.; Kim, H. J.; Kim, G. T.; Chang, I. S.; Lee, J.; Phung, N. T. *Appl. Microbiol. Biotechnol.* **2004**, *63*, 672–681.

(12) Lee, J.; Phung, N. T.; Chang, I. S.; Kim, B. H.; Sung, H. C. *FEMS Microbiol. Lett.* **2003**, *223*, 185–191.

(13) Phung, N. T.; Lee, J.; Kang, K. H.; Chang, I. S.; Gadd, G. M.; Kim, B. H. *FEMS Microbiol. Lett.* **2004**, *233*, 77–82.

(14) Liu, H.; Cheng, S.; Logan, B. E. *Environ. Sci. Technol.* **2005**, *39*, 658–662.

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However, many species known as non-EABs were also present in MFCs fed with acetate.[12]

Formate is also a nonfermentable fatty acid that can be metabolized directly by bacteria.[15] It is also known both as an intermediate and as a precursor in the synthesis of acetate from a single carbon source by acetogenic bacteria.[16,17] This synthesis process is known as the acetyl-CoA pathway.[18–20]

To our knowledge, there is no report on the use of formate as a fuel for MFCs. In this study, we examined the performance of MFCs enriched with formate.

2. Experimental Section

2.1. MFC Construction and Operation. The C-type mediatorless MFC[21] was used in this study. The anode and cathode compartments (working volume of 5 mL each) were separated by a Nafion450 cation exchange membrane (DuPont, Wilmington, DE). Each compartment contained two pieces ($45 \times 10 \times 5$ mm^3) of graphite felt (GF series, Electrosynthesis Co., Lancaster, NY) serving as electrodes. The platinum wires (0.5 mm diameter) were connected between electrodes and the electric ports. The wires were connected to an external resistance of 10 Ω during operation, and a digital multimeter (Keithley 2700, Keithley, Cleveland, OH). MFCs were operated continuously by feeding medium to the anode compartment via an up-flow pump-delivery system. Concurrently, the cathode compartment was continuously fed with air-saturated distilled water. Peristaltic pumps (Watson-Marlow, Campel, U.K.) were used to feed MFCs at the desired flow rate. Three MFCs were installed in a temperature-controlled chamber (30 °C).

2.2. Inoculum and Medium. Anaerobic digested fluid (ADF) was collected from a sewage treatment plant (Gwangju, Korea) and used as an inoculum source. The fresh inoculum was prepared by diluting ADF (50%) with medium containing formate (20 mM). After inoculation, bacteria in the anode chambers were starved for 1 day before being fed continuously with medium (with formate). The medium was prepared by dissolving inorganic salts and trace minerals into phosphate buffer (25 mM, pH 7.0) as described previously[12] and adding 20 mM sodium formate (Sigma, St. Louis, MO). The medium was autoclaved at 121 °C (15 min) and cooled under nitrogen gas atmosphere before being supplied to the MFCs. During operation, a gas-tight bag (SKC, Eight Four, PA) containing oxygen-free nitrogen was connected to maintain anaerobic conditions in the medium bottle. Acetate medium for the acetate-fed MFCs was prepared in similar manner using acetate fuel at 5 mM final concentration.

2.3. Instrumentation and Analyses. The potential development between anode and cathode was measured using a digital multimeter (Keithley 2700) and recorded on a personal computer through a data acquisition system (ExceLINX, Keithley Co.) at 5 min interval. The measured potential was converted to current according to Ohm's law [potential (V) = current (I) × resistance (R)]. Current was also converted to coulombs (C) using the equation current (A) = coulomb (C)/time (sec)]. Coulombic yield was calculated by dividing observed coulombs by theoretical coulombs, which was determined from the amount of substrate consumed. Chemical oxygen demand (COD) was observed by using a $COD_{(Cr)}LR$ Kit (Humas Co., Ltd., Daejeon, Korea). One milligram of COD was equivalent to 12 C. Fatty acids were analyzed by HPLC using an Aminex HPX 87H column (Bio-Rad Laboratories, Hercules, CA) equipped a UV detector (at 210 nm). All experiments were performed in triplicate, and the mean values were presented.

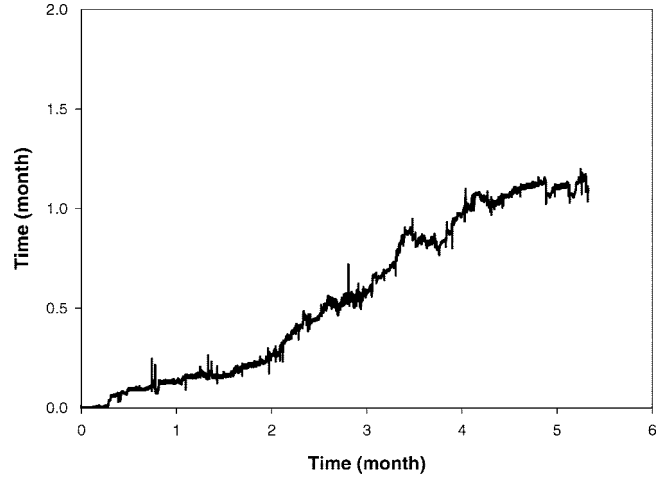

Figure 1. Typical current generation from MFCs using formate as fuel.

2.4. Microbial Diversity Studies. The graphite felt electrodes from the anode compartment of the MFCs were used for DNA extraction. Genomic DNA from ADF, which was used as inoculum, was also extracted using a PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc. Carlsbad, CA) according to the manufacturer's instruction. Small rDNA (16S rDNA) amplification and PCR-DGGE analysis were performed as previously described.[12,13] The bands on the DGGE gel were carefully excised with a razor blade under UV illumination, then transferred to a tube containing 50 μL of Tris-HCl (10 mM, pH 8.0) and incubated overnight at 4 °C to allow the diffusion of the DNA. Supernatants were used as the template for further PCR reaction to re-amplify the expected DNA from the DGGE bands. A 543r primer and a modified 341f primer (M341f, 5′-TAC GGG AGG CGA CAG-3′) were used in this reaction. The DGGE bands were cloned into pGEM-T Easy Vector System I (Promega, Madison, WI) and transformed into competent *Escherichia coli* DH5α cells (TaKaRa, Japan). Plasmids containing the hypervariable sequence excised from the DGGE gel were sent for sequencing (SolGent Co., Ltd., Korea). Sequences were analyzed using the MEGA version 3.1 program and compared with those in the BLAST GenBank (http://www.ncbi.nlm.nih.gov/blast/). Species types and classes of similar clones were searched on the Deusch Sammlung von Mikroorganismen und Zellkuturen GmbH (DSMZ, Germany) Web site (http://www.dsmz.de/microorganisms/) and in Bergey's manual of Systematic Bacteriology (2001).

3. Results and Discussion

3.1. Performance of MFCs Fed with Formate. Figure 1 shows a typical potential development pattern on MFCs. The maximum closed circuit potential of approximately 10 mV, which is equivalent to 1 mA current, was developed in 4 months. When fuel feeding to the MFC was stopped, the current decreased gradually, and it regenerated immediately as the feeding was resumed. Repeated medium replacements were coupled with current increase, together with a stepwise fall in formate concentration in the effluents. When current was stably developed to 1 mA, the formate concentration of effluent ranged from 1.5 to 0.4 mM. These results suggested that the inoculum contained a formate utilizer and formate utilizer itself or EAB was capable of utilizing formate as a metabolite to transfer electrons to the electrode in the anode. Previous results showed that almost all MFCs using other fuels including carbohydrates (glucose) and fatty acids (acetate, propionate) were stably enriched within 3 weeks.[9,10,12] They also generated higher current (e.g., Lee et al.[12] observed a current of 1.4 mA after 2 weeks of operation with MFCs fueled with acetate). Because

(15) Ribbons, D. W.; Harrison, J. E.; Wadzinski, A. M. *Annu. Rev. Microbiol.* **1970**, *24*, 135–158.
(16) Kerby, R.; Niemczura, W.; Zeikus, J. G. *J. Bacteriol.* **1983**, *155*, 1208–1218.
(17) Ljungdahl, L. G.; Wood, H. G. In *Vitamin B12*; Dolphin, D.; Ed.; 1982; Vol. 2, pp 165–202.
(18) Ljungdahl, L. G. *Annu. Rev. Microbiol.* **1986**, *40*, 415–450.
(19) Muller, V. *Appl. Environ. Microbiol.* **2003**, *69*, 6345–6353.
(20) Stephen, W. R. *Crit. Rev. Biochem. Mol. Biol.* **2004**, *39*, 165–195.
(21) Kim, B. H., Chang, I. S.; Moon, H. In *Encyclopedia of sensors*; Grimes C. A., Dickey, E. C., Pishko, M. V., Eds.; American Scientific Publishers: Stevenson Ranch, CA, 2006; Vol. 6.

Table 1. Substrate Degradation and Coulombic Efficiency

anode medium	COD removal (%)	Coulombic yield (%)[a]	formate removal (%)	Coulombic yield (%)[b]
formate, 20 mM	83	6.5	92	5.3
formate, 20 mM, + azide, 0.1 mM	67	6.6	99	4.5
formate, 20 mM, + azide, 3 mM	50	8.3	75	6

[a] Calculation based on amount of COD removal. [b] Calculation based on amount of consumed formate fuel.

these MFCs were operated under similar conditions with respect to anode and cathode compartments, this retarded current development on the MFC with formate could be due to the nutritional characteristics of formate as fuel.

When the current stabilized, the influent and effluent solutions were analyzed for fatty acid and COD concentration. Table 1 shows that almost all of the formate (92%) was consumed but only 5.3% of the electrons available from this formate oxidation was recovered as electricity. There was a slightly higher Coulombic yield (6.5%) based on COD reduction (83%) than that of formate consumption, indicating the existence of other metabolites, probably by a formate utilizer. Significant concentrations of acetate ranging from 0.4 to 0.6 mM were also detected in effluent by HPLC. These acetate concentrations were equivalent to 8% to 12% of fuel, which matched the difference between the amount of formate removal and COD removal (92% − 83% = 9%).

3.2. DGGE and Sequence Analyses. MFC was dismantled and anode electrode was divided into two sections (bottom and top). The bottom section was close to the influent port, and the top section was close to the effluent port. Individually, genomic DNA of four samples—both sections, inoculum sludge, and anode electrode—of the acetate-fed MFC was extracted. After PCR-DGGE, the DGGE patterns were compared to see the difference in bacterial consortia (Figure 2). Different bands on the DGGE gel represented different bacterial genotypes. Figure 2 shows that DGGE patterns of both bottom and top samples are similar to that of inoculum sludge, both in the number of bands and in the intensity of the major bands. This suggests that not only formate- but also acetate-fed MFC systems were not specially enriched for the electrochemically active bacterial consortia. Most of the bacteria in the inoculum were still present in the anode electrode of the MFCs. However, there were some noticeable differences on the gels, for the DNA fragments that had a higher G+C content. The DNA bands of electrode samples in this region showed a higher intensity compared with those of the inoculum sample, suggesting that these bacteria were somewhat enriched in MFC systems. In addition, the DGGE gel of DNA sampled from the electrode fed with acetate (lane 4 in Figure 2) showed fewer bands than that of the sample extracted from the electrode fed with formate (lane 2 and 3 in Figure 2).

Interestingly, there was a unique band on lane 3 (top electrode fed with formate) that showed a similar migration distance compared with band 2 on lane 4 (fed with acetate). This band was not clearly detected on lane 2 (bottom electrode fed with formate). Probably, this band 2 was related to the bacteria involved in the consumption of acetate, which also presented in formate-fed MFCs. The bands 3, 4, and 5 were detected on formate samples (bottom, lane 2) at band intensities higher than (band 3 and 5) or similar to (band 4) those of inoculum sludge (lane 1). Two bands (band 3 and 5) showed higher intensities from the bottom section of the formate sample anode than from the top section and were almost nondetectable in acetate-fed MFCs. This indicated that bands 3 and 5 were not directly

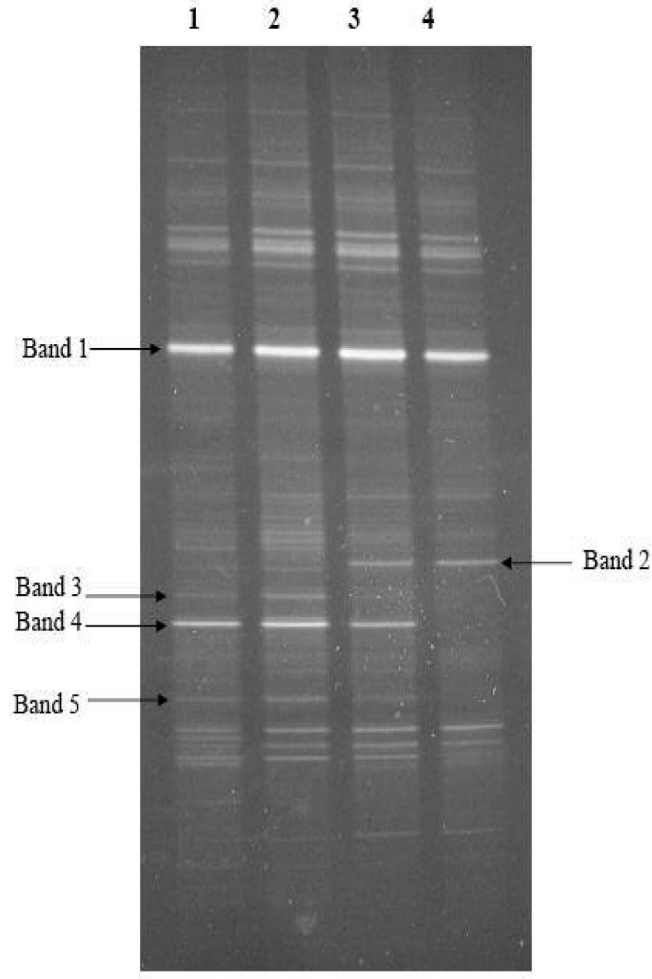

Figure 2. Comparison of bacteria diversity in the electrode of formate-enriched MFC with that in the electrode of acetate MFC and in the initial sludge: lane 1, initial sludge (inoculum); lane 2, bottom section of anode of formate-enriched MFC; lane 3, top section of anode of formate-enriched MFC; lane 4, anode of acetate-enriched MFC.

related to acetate catabolism. Band 4 was detected in inoculum and formate-fed samples but not detected in the acetate-fed sample. Band 1 may represent one of the dominant bacteria in the inoculum, which remained content in both formate- and acetate fed MFCs. Selected DGGE bands as show in Figure 2 were excised from the polyacrylamide gel for sequencing. The sequence analysis classified bacterial consortium representative for the individual DNA band detected on DGGE gel (Table 2).

As Sekiguchi et al.[22] reported, even though the excised DNA from the DGGE showed single band, the library consisted of several different sequences phylogenetically. The sequencing analyses corroborated the result observed previously from DGGE patterns and the performance of formate MFCs. Of all the phenotypes represented in the DGGE bands, an *Acetobacterium* sp. was commonly found in the entire DNA banding sample from electrodes of the formate-fed MFCs. This indicated the common existence of an *Acetobacterium* sp. in the formate-fed MFCs. *Acetobacterium* is known as a genus of Gram-negative acetogenic bacterium in the family of Eubacteriaceae[23] having the ability to synthesize acetate from single-carbon

(22) Sekiguchi, H.; Tomioka, N.; Nakahara, T.; Uchiyama, H. *Biotechnol. Lett.* **2001**, *23*, 1205–1208.

(23) Willems, A.; Collins, M. D. *Int. J. Syst. Bacteriol.* **1996**, *46*, 1083–1087.

Table 2. Sequencing Analysis Result of the DNA Sequences Excised from DGGE Bands

closest identification by BLAST search	accession no.	homology (%)[a]	class	percentage[b] of clones band 1	band 2	band 3	band 4	band 5
Arcobacter skirrowii	DQ464344	100	ε-proteobacteria	80	4.2	3.6		
Arcobacter butzleri	DQ464340	100						
Arcobacter cibarius	AJ607392	100						
Acetobacterium woodii	X96954	99	Clostridia	20	8.4	48.1	17.4	45.5
Acetobacterium malicum	X96957	99						
Acetobacterium submarinus	AY485791	99						
uncultured *Acinetobacter*	AF451251 EF114343	99	γ-proteobacteria		54			
iron-reducing bacterium	DQ676996 DQ677001	97	environmental sample			14.3	82.6	27.3
Geobacter sulfurreducens	AE017207	95	δ-proteobacteria			3.6		22.7
Geobacter metallireducens	CP000148							
uncultured Bacteroidetes bacterium	AM157621 AB03389	94	Bacteroidetes		16.7	26.8		
sulfate-reducing bacterium	AJ012601	95	δ-proteobacteria		12.5	3.6		4.5
uncultured Chloroflexi bacterium	EF029425 EF029259	98	Chloroflexi		4.2			

[a] Percentage of similarity between cloned hypervariable DNA sequence and closest relative in the NCBI database. [b] Percentage of similar hypervariable DNA sequence presented in a single DGGE band (n = 28–30).

compounds including formate. The synthesizing of acetate is performed under the acetyl-CoA pathway, which was named as the Wood/Ljungdahl pathway.[17,18] *Acetobacterium woodii* was well-known for that ability.[16] The acetate detected in the effluent could support acetogenic activity on formate-fed MFCs. Higher percentages of *Acetobacterium* sp. were found than other classes when the sequences of bands 3 and 5 were analyzed (46.4% and 45.5%, respectively). This result also correlated with the DGGE result, which showed bands 3 and 5 as unique bands in the electrode fed with formate. Specially, these two bands appeared at higher intensity at the bottom section of the electrode, which received a higher formate concentration than the top section.

Arcobacter is the genus of the family Campylobacteriaceae, which can grow in microaerobic conditions.[24,25] *Arcobacter* was found in the majority (80%) of phenotypes represented in band 1. This band was shown as a major band in the DGGE patterns of all the DNA samples. It was likely that these microaerophilic bacteria were widely present in the inoculum. Moreover, the anode compartment of the MFCs could have microaerobic conditions because of oxygen diffusing from cathode compartment of the MFCs.[26,27] Thus, during operation a remarkable amount of formate as well as acetate in the anode compartment might be consumed. This result was confirmed by the decreased COD removal as the anode medium was supplemented with azide (Table 1), which is an aerobic respiration inhibitor.[28]

Compared with the DGGE banding pattern observed with different inoculum sources utilized in other MFC studies,[9,10,12,13] this major band was not detected. Hence its bacterial consortium was not enriched during MFC operation. In other words, we could conclude that MFC performance also depended on the inoculum source.

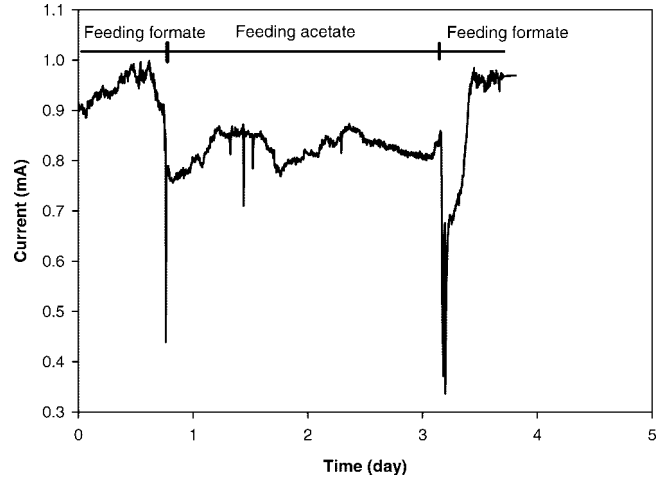

Figure 3. Current generation from formate MFCs fed with acetate fuel.

Results from the previous studies[2,4,5] showed the direct transfer of electrons to the electrode in *Geobacter* and many other DMRB such as *Shewanella putrefaciens*,[2,5] *Rhodoferax ferrireducens*,[7] and *Aeromonas hydrophyla*.[29] The previous studies of microbial diversity in acetate-fed MFCs, bacterial consortia revealed the common presence of *Geobacter* sp.[9,12] In this study, *Geobacter* sp. accounted for 3.6% and 22.7% of the DNA sequences from bands 3 and 5, respectively, while the genotype of iron-reducing bacteria was mainly detected in the sequence result of band 4, at a percentage of 82.6%. It is not clear that this band represents the bacterium capable of current production with formate oxidation.

The initial formate-fed MFCs were operated with introduction of medium containing acetate as COD of 100 mg/L to test whether these MFCs could utilize acetate as fuel. Figure 3 shows that MFCs produced electricity with a short retardation. However, the Coulombic yield was 20% based on COD removal. This low Coulombic efficiency could be due to higher fuel consumption rates of aerobic bacteria than those of bacterial consortia utilizing acetate to produce electricity. The short retardation might be the current generated from EABs consuming some formate fuel.

(24) Garrity, G. M.; Holt, J. G. *Bergey's Manual of Systematic Bacteriology*, 2nd ed.; Boone, D. R., Castenholz, R. W., Garrity, G. M., Eds.; Springer-Verlag Publishers: New York, 2001; pp 119–166.

(25) Vandamme, P.; Dewhirst, F. E.; Paster, B. J.; On, S. L. W. *Bergey's Manual of Systematic Bacteriology*, 2nd ed.; Brenner, D. J., Krieg, N. R., Staley, J. T., Garrity, G., Eds.; Springer-Verlag Publishers: New York, 2005; pp 1161–1164.

(26) Gil, G. C.; Chang, I. S.; Kim, B. H.; Kim, M.; Jang, J. K.; Park, H. S.; Kim, H. J. *Biosens. Bioelectron.* **2003**, *18*, 327–334.

(27) Pham, T. H.; Jang, J. K.; Chang, I. S.; Kim, B. H. *J. Microbiol. Biotechnol.* **2004**, *12*, 324–329.

(28) Chang, I. S.; Moon, H.; Jang, J. K.; Kim, B. H. *Biosens. Bioelectron.* **2005**, *20*, 1856–1859.

(29) Pham, C. A.; Jung, S. J.; Phung, N. T.; Lee, J.; Chang, I. S.; Kim, B. H.; Yi, H.; Chun, J. *FEMS Microbiol. Lett.* **2003**, *223*, 129–134.

Some other classes such as Bacteroidetes, γ-proteobacteria (*Acinetobacter*), and δ-proteobacteria (sulfate-reducing bacteria) were also detected, but as minor components of the community or only detected from the DNA of minor bands. These consortia were also found in previously studied bacteria communities in MFCs[9,12,13] as minority components of population. These findings suggest that these bacteria are widely distributed in the ecosystems with various fuel favors, and they can grow under the conditions in the anode compartment of an MFC system and then might be as fuel competitors to the EAB consortia.

4. Conclusions

Based on the results, we can theorize that acetogenic bacteria consume formate to synthesize acetate via the acetyl-CoA pathway, and acetate could be used to produce electricity by acetate-oxidizing EABs. However, both formate and acetate were easily oxidized by microaerobic bacteria such as an *Arcobacter* sp. without electricity production. To increase the efficiency of these MFCs fed with formate, undesirable bacterial communities should be inhibited. Aerobes could be controlled by using respiratory inhibitors such as azide or cyanide, which have been used in MFCs.[28] Alternatively, oxygen diffusion from cathode to anode compartments could be reduced by achieving further increases in cathode reaction with a catalyst that shows a high oxygen affinity to the electrode, not only through the use of precious metals (e.g., platinum) but also by using biocatalysts such as oxygen-reducing enzymes.

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