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Geochip-Based Functional Gene Analysis of Anodophilic Communities in Microbial Electrolysis Cells under Different Operational Modes

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A microbial electrolysis cell (MEC) is a bioelectrochemical system that can produce hydrogen from acetate at high hydrogen recoveries, but the composition and structure of the microbial communities in this system have not been extensively studied. We used a high throughput metagenomics technology (GeoChip) to examine the microbial community functional structure in MECs initially operated under different conditions. We found that startup conditions had little or no effect on reactor performance in terms of Coulombic efficiencies (CEs) and COD removals, somewhat greater effects on CO_2 and CH_4 production, and very large effects on hydrogen production. Hydrogen yields were generally higher for reactors that were always operated as MECs than those initially operated as MFCs. Hydrogen yields were nine times larger for MEC reactors with an applied voltage of 0.7 V (64%∼80% efficiencies) than 0.3 V (<7−8%), independent of startup conditions. GeoChip analysis revealed that the functional and phylogenetic diversity of MEC microbial communities after 4 months was quite high despite the use of only a single substrate (acetate). MECs with the largest hydrogen yields had the highest microbial diversity. Multivariate analyses showed that communities that developed in the MECs were well separated from those present under startup conditions, indicating reactor operation altered microbial community composition. Community shifts based on a Mantel test were significantly related to CEs and COD removals in these reactors, suggesting that there were significant changes in microbial community composition as a result of conditions that affected MEC performance. Common well-known exoelectrogenic bacteria (e.g., *Geobacter*, *Shewanella*, *Desulfovibrio*, and *Anaeromyxobacter*) were found in these systems, but their importance in determining reactor functional performance was not supported with a high confidence in our statistical analysis.

Introduction

Biohydrogen can be produced by different biological processes such as photosynthesis, photofermentation, and dark fermentation (*1, 2*). Dark fermentation-based H_2 production is one of the most efficient approaches relative to commercial applications, and thus intensive studies have been carried out since the 1960s on using this approach (*2*). However, the hydrogen yield of dark fermentation has a theoretical potential of only 4 mol H_2/mol glucose ($^1/_3$ of the possible stiochiometric yield), and in practice yields of only 2−3 mol H_2/mol glucose (*3*). The remaining organic matter is converted to acetate and other fermentation products (primarily butyrate and alcohols), which are referred to as "dead end" products because high yields of hydrogen are not thermodynamically possible under practical conditions for these compounds. A key challenge in renewable energy generation is to identify methods to efficiently convert these dead end products into hydrogen.

A new process was called electrohydrogenesis was recently developed that is based on using exoelectrogenic bacteria in devices called microbial electrolysis cell (MECs). Using an MEC it is possible to convert fermentation end products to H_2 at yields of 3−4 mol H_2/mol for acetate, and 8.2 mol H_2 /mol for cellulose (68% of the stoichiometric limit) (*4*). The energy efficiency with cellulose was 268% relative to the electrical energy required, and 63% overall. The overall energy yield using only acetate was 82%. Although MECs look promising for efficient H_2 production, very little is known about microbial populations that develop in these systems.

Most microbial communities present in MFCs and MECs have previously been examined using denaturing gradient gel electrophoresis (DGGE) to identify key species present in these systems (*5*−*7*). While this shows relative abundances of different strains, it does not provide any information on the functional capabilities of these communities. Recently, high throughput genomic technologies such as pyrosequencing, PhyloChip, and GeoChip have been developed as alternative methods for analyzing microbial community functional structures (*8*). A GeoChip can identify a variety of geochemically important functional genes, including those related to carbon, nitrogen, and sulfur cycling, phosphorus utilization, metal resistance, and organic pollutant remediation (*9, 10*). This type of array is useful in profiling changes in microbial community functional structures under different conditions, but they have not previously been applied to MECs.

Exoelectrogenic bacteria using attach to the anode and form a biofilm (*11*−*14*). Thus, we hypothesize that the microorganisms that develop in this biofilm are linked to operational conditions that have significant impacts on reactor performance. In this study, we used GeoChips to address the following questions: What is the functional diversity of microbial communities in these bioreactors? Do operational conditions affect microbial community functional structure? Do the differences in microbial communities significantly affect the performance of MECs for hydrogen

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production? Our results indicate that the microbial communities in these reactors are functionally diverse, and that the operational conditions such as startup mode and applied voltage can affect microbial community functional structure.

Materials and Methods

Reactor Setup and Operational Conditions. Experiments were conducted using single chamber reactors made of polycarbonate as previously described (*11*). The total volume was 43 mL, consisting of a 28.5 mL chamber (3 cm inner diameter, 4 cm long) and a gas collection tube attached to the top of the reactor (1.6 cm inner diameter, 7.5 cm length; 14.5 mL capacity). The anodes and cathodes used in this study were described previously (*15*). The cathode was placed on the side opposite the anode brush. For MFC operation, the cathode was exposed to air on one side and the liquid medium on the other. For MEC operation, the cathode was placed against the sealed wall of the reactor.

Six single chamber reactors were set up and inoculated using domestic wastewater (primary clarifier effluent) from the Pennsylvania State University Wastewater Treatment Plant with acetate as the carbon source. The medium contained a phosphate buffer (PBS, 50 mM; pH 7.0; conductivity = 7.5 mS cm^{-1}) and nutrient solution (NH_4Cl, 310 mg L^{-1}; KCl, 130 mg L^{-1}; with a trace nutrient medium), and sodium acetate (1.5 g L^{-1}). Reactors were divided into two groups for studying the development of microbial communities. Two reactors (nos. 1 and 2) were first operated as MFCs (1000 Ω external resistance), with a third reactor (no. 3) operated in open circuit mode. Two other reactors were always operated in MEC mode at fixed applied voltages of 0.3 V (no. 4) or 0.7 V (no. 5) (model 3645A; Circuit Specialists, Inc.), with an additional reactor (no. 6) maintained in open circuit mode (Supporting Information (SI) Table S1). The two control reactors differ in the cathode either being completely sealed (no. 6) or exposed to air (no. 3).

During startup, all reactors were fed a 50:50 mixture of wastewater and buffer (two, 24-h batch cycles). The reactors were then switched to the acetate medium and operated as MFCs or MECs for one month. Next, the MFCs were switched to MEC mode after replacing the cathodes, and operated at applied voltages of 0.3 V (no. 1), 0.7 V (no. 2), or with an open circuit (no. 3). New cathodes were also used for the three MEC reactors (reactors no. 4−6) to ensure comparable cathode potentials. Reactors were operated in fed batch mode, with the medium replaced when voltage decreased below 0.1 V for MFCs, or when current decreased below 0.2 mA for MECs. After refilling the reactors, the chambers were purged using N_2 gas (99.998%) for 20 min. All tests were conducted at room temperature.

Voltages across a resistor (1000 Ω for MFCs, 1.3 Ω for MECs) were measured using a multimeter (model 2700; Keithley Instruments). Chemical oxygen demand (COD) was performed at the start and end of each cycle according to standard methods (TNT plus COD Reagent; HACH Company). Gases were collected in gas bags (0.1 L capacity; Cali-5-Bond, Calibrated Instruments) for analysis. Gas samples were taken from the reactor headspace and gas bag using a gastight syringe (250 μL, Hamilton Sample-lock Syringe). Analysis of the gas (H_2, CO_2, and CH_4) was conducted using gas chromatography (*11*). At the end of the experiment, biofilms were scraped from anode brushes for community analysis. The samples were stored at −80 °C prior to DNA extraction.

DNA Extraction, Amplification, Labeling and Hybridization. DNA was isolated from the biofilm samples using a freeze-grinding method (*16*). DNA (100 ng) from each sample was amplified in triplicate using the Templiphi kit (GE Healthcare) following established procedures and modifications by adding spermidine (0.1 mM) and single-stranded binding protein (260 ng μL^{-1}) (*9*). Amplified DNA (∼28 μL) was labeled with Cy-5 and purified using columns (QIAquick purification kit; Qiagen) according to the manufacturer's instructions, and dried at 45 °C for 45 min (SpeedVac, ThermoSavant). Samples were hybridized to GeoChip 3.0 for 12 h at 42 °C and 50% formamide (*17*).

Data Analysis. After hybridization, GeoChips were scanned (ScanArray Express Microarray Scanner, Perkin-Elmer) and analyzed using the Imagene software (6.0 premium version, Biodiscovery, El Segundo, CA). Spots with signal-to-noise ratios (SNR) < 2, outliers (standard deviation, SD > 3), and poor spots were removed. Data normalization was based on the method of mean signal intensity across all genes (*18*), and any gene with more than $^1/_3$ of the probe spots hybridized was considered positive (*19*).

Functional gene diversity was calculated using Shannon-Weaver index (H) with the freely available software (http://www2.biology.ualberta.ca/jbrzusto/krebswin.html). Cluster analysis was performed using the pairwise average-linkage hierarchical clustering algorithm in the CLUSTER software (http://rana.stanford.edu), and the results of hierarchical clustering were visualized using the TREEVIEW software (http://rana.stanford.edu/). PCA was performed using PC-ORD (Version 5.0 MjM Software). A Mantel test (*20*) was used for inferring associations between functional gene structure and bioreactor performance. The matrices required for Mantel tests were constructed based on Euclidean distance measurements, that is, dissimilarity matrices of functional gene abundance and the functional measurements of the bioreactors. The majority of analyses were done by functions in vegan packages (*21*) with some additional code utilizing vegan package functions installed in our GeoChip analysis pipeline.

Results

Bioreactor Performance. Five days after inoculation, all closed-circuit reactors produced current. After another six days of operation, all reactors demonstrated reproducible cycles of voltage and current generation (SI Figure S1 and S2). The performance of reactors nos. 1 and 2 were not significantly different ($p < 0.1$, t test) during the month they were operated as MFCs producing an average peak voltage of 571 ± 4 mV (1000 Ω; both reactors), an average peak current of 0.57 ± 0.01 mA, and CEs of 22 ± 4% (data from the last five cycles, over 10 days) (SI Figure S4). The open circuit potential was constant (785 mV) for the MFC control reactor no. 3

Reactors always operated as MECs produced maximum currents of 1.1 ± 0.4 mA at 0.3 V (no. 4) and 7.4 ± 0.2 mA at 0.7 V (no. 5). CEs were much higher than those of the MFCs due to the lack of exposure of the cathode to air, with CEs of 88 ± 2% (0.3 V) and 90 ± 4% (0.7 V). The overall conversion efficiency of acetate into hydrogen was 80 ± 5% at an applied voltage of 0.7 V, but only 4.9% at 0.3 V. When MFCs (nos. 1 and 2) were switched to MEC mode, there was immediate current generation and hydrogen production in the first cycle. The peak currents produced by these reactors were similar to the levels produced by reactors always operated in MEC mode at corresponding applied voltages (SI Figure S3). The CEs of the reactors switched to MEC mode were increased to 90% (no. 1) and 95% (no. 2) (SI Table S1). At 0.3 V, the hydrogen yield of reactor no. 1 was 7.0%, similar to the 7.5% yield of reactor no. 4. The hydrogen yield became significantly larger at 0.7 V (Table 1; $p = 0.008$), with a yield of 64.3% (no. 2) compared to 72.1% for the reactor always operated as an MEC (no. 5) (Table 1).

Much less CH_4 was detected during MEC operation for the reactors initially operated as MFCs, when compared on the basis of applied voltage. In addition, less CH_4 and much higher hydrogen yields were obtained for reactors operated

TABLE 1. Gene Overlap (Unshaded Number and Percentages), Gene Uniqueness (Shaded Gene Number and Percentages), And Diversity Indices for Each Sample

reactors[a]	no. 1	no. 2	no. 3	no. 4	no. 5	no. 6
no. 1	86(6.4%)	784(41.7%)	847(42.0%)	1039(47.6%)	1164(34.6%)	1028(32.4%)
no. 2		112(8.5%)	850(42.9%)	1004(45.9%)	1086(31.8%)	998(31.4%)
no. 3			158(10.4%)	1128(49.9%)	1258(36.6%)	1193(37.5%)
no. 4				125(6.7%)	1628(47.4%)	1402(42.1%)
no. 5					778(24.4%)	1950(47.6%)
no. 6						738(25.8%)
no. of genes detected	1347	1316	1515	1875	3185	2858
shannon diversity	6.9	6.9	7.0	7.2	7.8	7.7
hydrogen[b] yield (%)	7.0	64.3 ± 3.3	na	7.5 ± 3.5	72.1 ± 3.6	na

[a] These reactors were operated under different startup conditions for 1.5 months and then run under MEC mode for 2.5 months. Different reactors represented different startup conditions: no. 1, MFC to MEC with 0.3 V; no. 2, MFC to MEC with 0.7 V; no. 3, MFC to MEC without voltage (control); no. 4, MEC with 0.3 V; no. 5, MEC with 0.7 V; and no. 6, MEC control without voltage (control). The diversity data was obtained with the biofilm communities using GeoChips at the end of experiments.

at 0.7 V than 0.3 V (SI Table S1). The highest CO_2 concentration in the gas was obtained for reactor no. 2 at 0.7 V, along with lower hydrogen yields and less CH_4 (Table 1, SI Table S1).

These results indicate startup conditions had little or no effect on CEs or COD removal (SI Table S1). Considerable differences were observed for CO_2 and CH_4 production among these reactors, but these differences were smaller than those associated with hydrogen production, indicating that the startup conditions had significant effects mainly on hydrogen production. The hydrogen yields in the reactors first operated as MFCs (nos. 1 or 2) were generally lower than those always operated as MECs (nos. 4 or 5). In addition, the applied voltages used in MEC tests had larger impacts on hydrogen yield, with 9 times more hydrogen produced at 0.7 V than at 0.3 V, independent of start up modes.

Overall Functional Gene Diversity of Microbial Communities. To understand whether startup conditions affected community composition and structure, the biofilm communities in the MECs were analyzed at the end of the experiments using GeoChips. A total of 4589 genes (18.7% of all probes) showed significant hybridization with at least one of the six samples. The number of the genes detected varied significantly across these samples, ranging from 1316 to 3185 genes (Table 1). The diversity of these communities varied considerably based on the Shannon−Weaver index. In general, MECs initially operated as MFCs had less genes detected and had a lower diversity index than those continuously operated as MECs. For example, the richness and diversity of reactor no. 2 (initially operated as an MFC) was substantially lower than those in reactor no. 5 (always operated as an MEC). An average of 40.8% genes (range of 31−50%) were shared among the reactors (Table 1). A total of 576 genes (12.5%) were shared across all samples. The percentage of unique genes varied from 6−26%, which is relatively high considering that a single inoculum source was used for all reactors.

To determine whether startup conditions affect the overall patterns of microbial community functional structure, PCA was performed based all detected functional genes. Principal components 1 and 2 explained 35.1% and 25.3% of the total community variations, respectively. As shown in Figure 1, the two control samples (nos. 3 and 6) (no applied voltages) were clustered together and were well separated from those reactors that had applied voltages. Reactors operated under similar startup conditions (nos. 1 and 2, and nos. 4 and 5) were also clustered and well separated from each other (Figure 1). These results show that initial operational conditions had a clear effect on the community functional structure in these MECs.

All major functional gene groups included in the GeoChip were detected in all samples, but their relative abundance

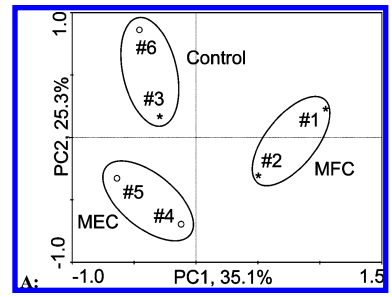

FIGURE 1. Principal component analysis (PCA) of communities from different MEC-reactors based on functional genes detected.

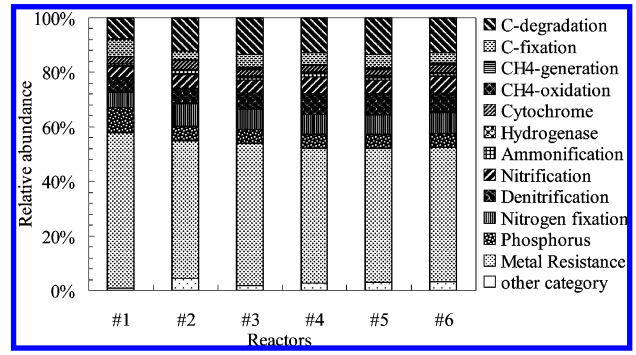

FIGURE 2. Relative abundance of various functional groups detected across different reactors.

varied considerably (Figure 2). For example, genes related to metal resistance were highly enriched, and accounted for 50−60% of all detected genes (Figure 2). Functional genes related to complex carbon utilization accounted for 7.8−13.3% of all detected genes (Figure 2), with smaller percentages of genes related to N cycling processes such as N fixation (5.5−8.4%), nitrification (4.1−6.3%), denitrification (5.4−7.8%), and P utilization (5−9%). Genes involved in cell respiration and possibly metal reduction (*c*-type cytochromes) accounted for about 2.5−4% of identified genes, while those related to methanogenesis genes accounted for 0−0.9%. The overall relative abundance of the various gene groups remained very similar across different reactors, but the diversity of microbial functional gene categories appears to be slightly higher in control reactors (nos. 3 and 6) than the other reactors.

Changes in Individual Functional Genes/Populations. To visualize how the functional genes changed among the different reactors, a cluster analysis was performed on detected genes (Figure 3). Consistent with PCA results, the cluster analysis also showed that the control reactors (nos.

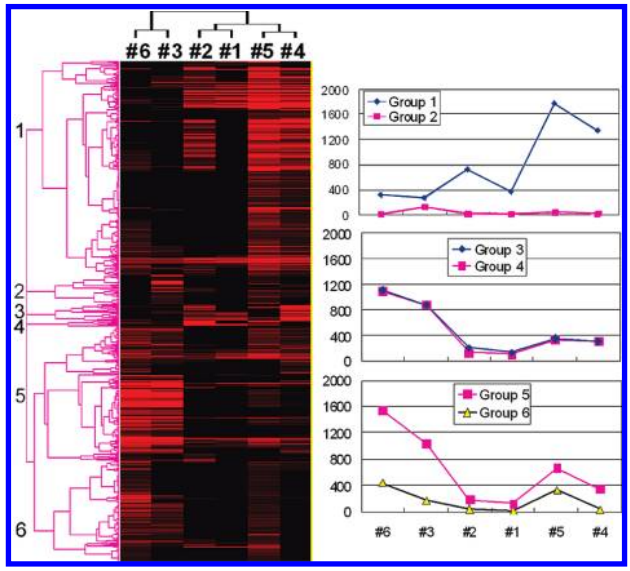

FIGURE 3. Hierarchical cluster analysis of genes based on hybridization signals. The figure was generated using CLUSTER and visualized with TREEVIEW. Black represents no hybridization above background level and red represents positive hybridization. The color intensity indicates differences in hybridization patters. The numbers equal groupings found among the hybridization patterns. Average signal intensities of these groups for each reactor are shown on the right.

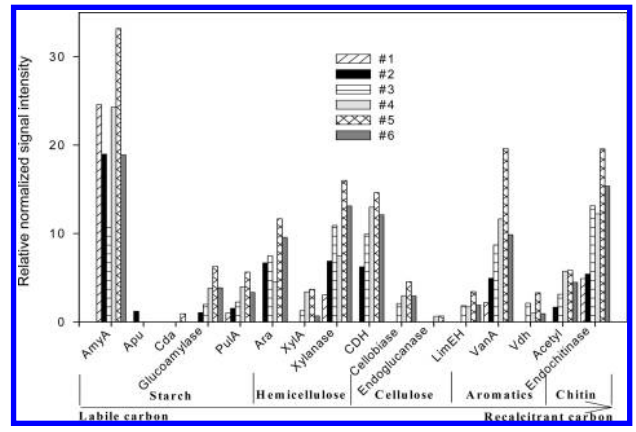

FIGURE 4. Relative abundance of the genes involved in complex carbon degradation among different MEC-reactors. Genes are arranged from labile carbon to recalcitrant carbon and their targeting carbons to degradation are listed below.

3 and 6) similar to each other and were well separated from other reactors (nos. 1 and 2, compared with nos. 4 and 5). The overall diversity and abundance of the functional genes detected in MECs initially operated as MFCs (nos. 1 and 2) were substantially lower than the reactors always operated as MECs (Figure 3).

A total of six different patterns of gene clustering were observed across these reactors. The most obvious pattern was Group 1 (666 genes, 32.3%) whose members were abundant across the treatment reactors (Figure 3). The genes in this group were from functional categories involving: cytochromes; nitrification, nitrogen fixation, and denitrification; phosphate utilization; and degradation of starch, glucose, and cellulose as well as many other organic contaminants; and metal resistance. The next obvious group (Group 5, 420 genes, 26.9%), included members mostly present in the control samples. This group also consisted of most of the genes belonging to the functional categories identified for group 6. Two of the other distinct patterns (Group 3, 4.6%; and Group 4, 0.8%) were also more abundant in the control reactors, but the genes in this group were related more to metal resistance, denitrification, phosphate utilization, and degradation of starch and aromatics. Other groups (Groups 2, 6) were present in low abundances (Figure 3).

Genes involved in complex carbon degradation were specifically examined in this study in order to better understand microbial diversity in these communities. A variety of carbon degradation genes were detected among these reactors (Figure 4), including amylase, xylanase, and endochitinase. Similar to overall diversity patterns, genes involved in carbon degradation were also highly diverse and the relative abundance of these genes varied considerably among the reactors. Reactor no. 5, which had the highest hydrogen yield, had the highest abundance for all detected genes involved in complex carbon degradation. This suggests that the capability for using a variety of complex carbon sources was a useful capability for successful growth in MECs with high hydrogen production.

Cytochrome genes (*22*−*24*) were also specifically analyzed as they are important for understanding cell respiration using an anode (Figure 5). GeoChip 3.0 contains probes from several metal reducing bacteria. A total of 75 (2.9%) *c*-type cytochrome genes were detected in these reactors, with genes from *Geobacter sulfurreducens* (3996436) and *G. metallireducens* (78221556) highly abundant in all reactors. The cytochrome genes from *Desulfovibrio* and *Rhodobacter* species were the next abundant genera in MECs. Cytochrome genes from other metal reducing bacteria such as *Shewanella* and *Anaeromyxobacter* were detected, but were low in abundance. The cytochromes MtrC (OmcB) and OmcA, which are outer membrane associated metal reductases, have been shown to be involved in electron transport in MFCs (*25*). OmcA/MtrC sequences from *Shewanella* (114563802, 127513453, 157374666) and *Geobacter* (39995699, 39996436, 39997094) were detected in the reactors. In addition, flavocytochromes, which act as electron shuttles were detected from *Anaeromyxobacter dehalogenans* (86158518) and *Shewanella* spp. (126172508, 127511210, 157960169, 157373414, 117922435, 157377310). Other cytochrome genes detected include CoxB/CtaC (cytochrome c oxidase involved in oxidative phopshorylation), DmsE (involved in DMSO reduction), ExaB (PedF, conversion of 2-phenylethylamine and 2-phenylethanol to phenylacetic acid), MopB (TMAO reductase), NapC/NirT (nitrate, nitrite, metal reduction), P450 (oxidation of organic contaminants), PetC (ubiquinol: cytochrome oxidase), NapB and NrfH (both nitrate reduction). The distribution of *c*-type cytochrome genes varied substantially among different reactors, with reactor nos. 2 and 5 having more abundant c-type cytochrome genes than other reactors. Interestingly, *Shewanella loihica,* and *S. denitrificans* were only observed in reactors no. 2 and/or no. 5 (Figure 5).

Relationships between Community Functional Structure and Bioreactor Performance. To understand whether the changes of community structure were important to bioreactor performance, Mantel tests were performed between community functional structure and each of the six reactor performance variables (CE, COD removal, H_2, CH_4, and CO_2). There was a significant correlation between CE and community composition ($r = 0.84$, $P = 0.025$), and COD removal and carbon degradation ($r = 0.84$, $P = 0.035$) with community structure. While the correlation between microbial community and CO_2 production was marginal ($r = 0.44$, $P = 0.09$), there were no significant relationships between community functional structure and hydrogen production ($r = 0.09$, $P = 0.158$) or CH_4 yield ($r = 0.23$, $P =$

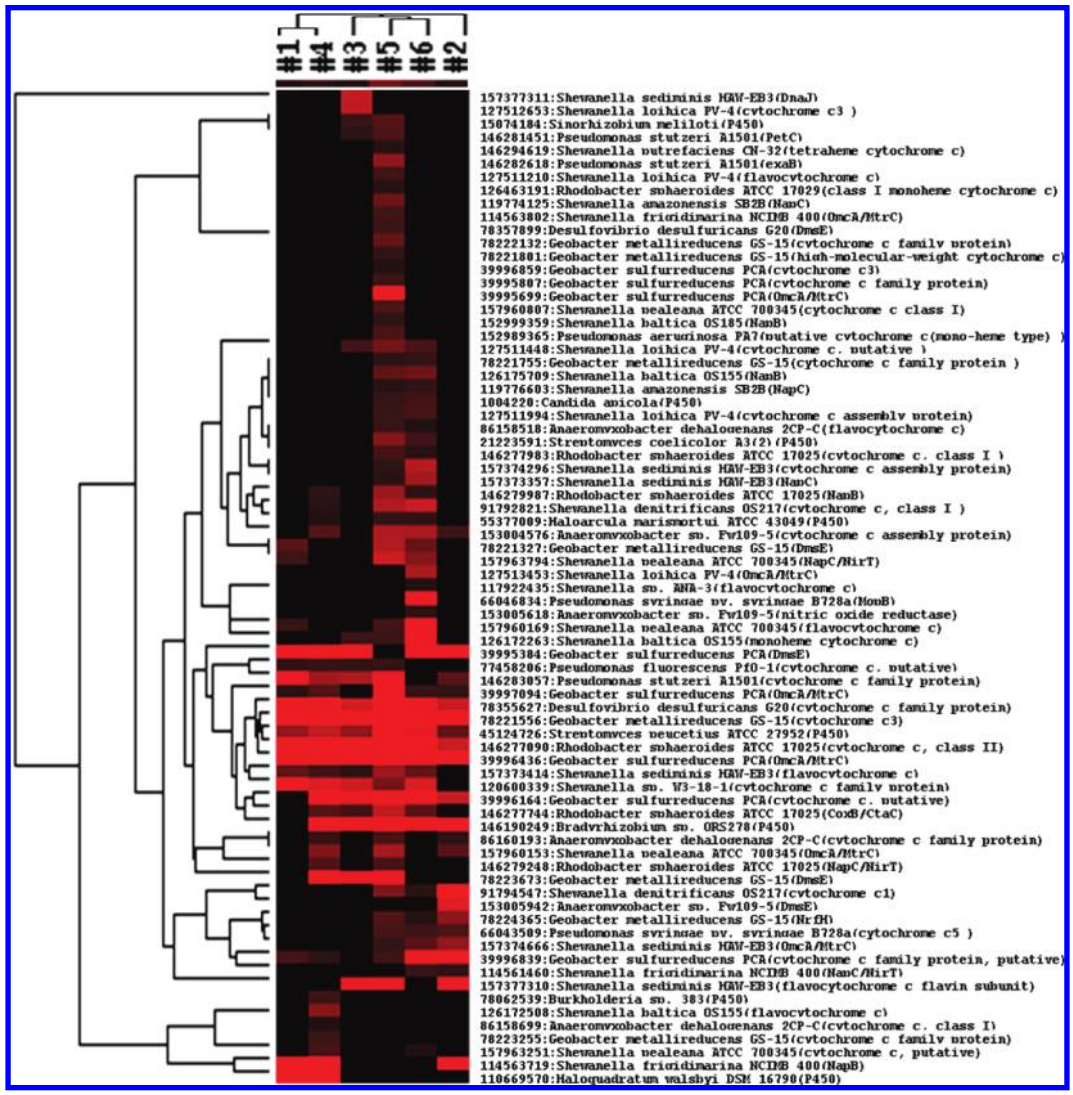

FIGURE 5. Hierarchical cluster analysis of c-type cytochrome genes. See Figure 3 legend for explanation.

0.175). Also, there were no significant correlations between each of the six reactor functional parameters and individual microbial populations, including *Geobacter*, *Shewanella*, *Desulfovibrio*, and *Pseudomonas* (data not shown).

Discussion

Although MECs and MFCs operate on the same principle of electron transport to the anode (*26, 27*), CEs are usually much higher in MECs than MFCs because oxygen is not used as the electron acceptor at the cathode. This difference the availability of oxygen can produce microbial communities with different functional capabilities. In addition, the high concentration of hydrogen gas in MECs can stimulate the growth of hydrogenotrophic microorganisms (*11, 28*). GeoChip analysis showed that startup conditions for the MECs (MFC first, or only MEC) significantly altered microbial community composition and structure. The initial start up of the MEC as an MFC also significantly reduced microbial community richness and diversity by the evaluation methods used here.

Since only acetate was used as a carbon source for these reactors, it was expected (and it was observed) that nonacetate utilizing bacteria such as *Shewanella* (under anoxic conditions) would not develop as significant members of the microbial community. Despite the use of a single substrate, however, GeoChip analysis revealed a relatively high functional and phylogenetic diversity of microorganisms. The diversity of the community may be maintained through utilization of excreted compounds by electrogenic bacteria, or by growth on the biomass of other cells by nonacetate utilizing bacteria. Such speculation is supported by the presence of many functional genes involved in degrading complex carbon sources. Further mechanistic studies on the relationships among the specific substrate, cellular biomass turnover, and microbial community functional structure and reactor performance are needed to better understand the presence of such a diverse community in these bioelectrochemical systems.

It has been shown that specific cytochromes and associated proteins have essential roles (*29, 30*) in extracellular electron transfer to electrodes and iron for certain *Shewanella* (*25*) and *Geobacter* (*31*) strains. Parts of certain cytochrome genes (365 probes) are targeted in GeoChip 3.0, and these cover the most well-known genes used by exoelectrogenic microorganisms. The communities present in the MECs, as inferred from the presence of specific cytochrome genes, showed the presence of several known exoelectrogenic genera such as *Geobacter* (*24*), *Shewanella* (*32*), *Desulfovibrio* (*33*), and *Anaeromyxobacter*. It is not possible to tell what role these bacteria served in current generation, although certainly *Geobacter* species were more abundant here than other exoelectrogens, and various

strains are well-known to produce high power densities in MFCs. While certain *Shewanella* species were found in these systems as well, and they are capable of growth using hydrogen, their abundance was low compared to *Geobacter* as expected based on the use of acetate as the sole carbon source.

Pseudomonas species are generally found to be abundant in MFCs and MECs (*6, 34*), despite reports that they produce low power densities in MFCs. These bacteria respire in an MFC using self-produced mediators, such as pycocyanin and related compounds, which can be used by other microorganisms for respiration with the anode (*6*). GeoChip analysis indicated that *Pseudomonas* species were abundant in these reactors examined here. Reactor no. 5, which had the highest CE and hydrogen conversion, also had the most abundant *Pseudomonas* populations. *Pseudomonas* spp. can use a wide and complex range of sources, and they also may be important for oxygen scavenging and maintaining anaerobic environments as some exoelectrogenic strains are obligate anaerobes.

Linking community structure to function is a fundamental issue in ecology (*35, 36*). Although microorganisms play important roles in various ecosystem processes, establishing the mechanistic linkages between microbial diversity and ecosystem functioning is very difficult because microbial community is usually very complex, especially for natural environments such as soils (*37, 38*). Bioreactors generally have simpler community structures, making it possible to more easily establish cause-and-effect relationships due to the ability to control system parameters, allow direct monitoring and data collection, and to conduct replicated experiments (*39*). The results presented in this study indicated that microbial community functional structure played an important role in certain bioreactor performance parameters, such as CE and COD removal, but not in others such as hydrogen productions. Statistical disassociation between community functional structure and the yields of hydrogen or methane could be due to small number of sample sizes or more highly variable parameters in comparison with other reactor parameters. Additional exploration of the communities in MECs, with clearer variations in performance parameters, will allow us to establish more direct cause-and-effect relationships between community structure and function.

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Supporting Information Available

Table S1 and Figures S1−S4. This material is available free of charge via the Internet at http://pubs.acs.org.

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