Environmental Science & Technology

Article

pubs.acs.org/est

Treatment of Alcohol Distillery Wastewater Using a Bacteroidetes-Dominant Thermophilic Microbial Fuel Cell

Phuc Thi Ha,[†] Tae Kwon Lee,[‡,§] Bruce E. Rittmann,[∥] Joonhong Park,[‡,§] and In Seop Chang*[,†]

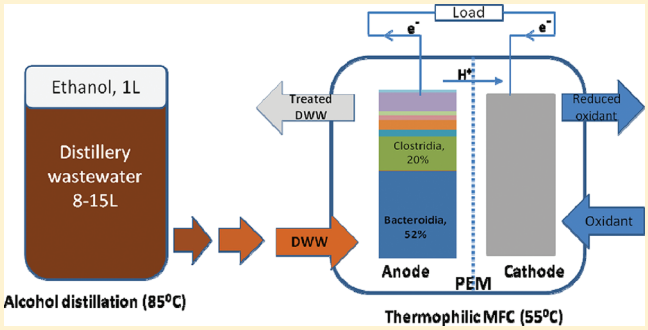
[†]School of Environmental Science and Engineering, Gwangju Institute of Science and Technology (GIST), 261 Cheomdan-gwagiro, Buk-gu, Gwangju 500-712, Korea

[‡]School of Civil and Environmental Engineering and [§]WCU Center for Green Metagenomics, Yonsei University, 262 Sungsanro, Seodaemungu, Seoul 120-749, Korea

[∥]Swette Center for Environmental Biotechnology, Biodesign Institute, Arizona State University, Tempe, Arizona 85287-5701, United States

Supporting Information

ABSTRACT: Simultaneous electricity generation and distillery wastewater (DWW) treatment were accomplished using a thermophilic microbial fuel cell (MFC). The results suggest that thermophilic MFCs, which require less energy for cooling the DWW, can achieve high efficiency for electricity generation and also reduce sulfate along with oxidizing complex organic substrates. The generated current density (2.3 A/m^2) and power density (up to 1.0 W/m^2) were higher than previous wastewater-treating MFCs. The significance of the high Coulombic efficiency (CE; up to 89%) indicated that electrical current was the most significant electron sink in thermophilic MFCs. Bacterial diversity based on pyrosequencing of the 16S rRNA gene revealed that known Deferribacteres and Firmicutes members were not dominant in the thermophilic MFC fed with DWW; instead, uncharacterized Bacteroidetes thermophiles were up to 52% of the total reads in the anode biofilm. Despite the complexity of the DWW, one single bacterial sequence (OTU D1) close to an uncultured *Bacteriodetes* bacterium became predominant, up to almost 40% of total reads. The proliferation of the D1 species was concurrent with high electricity generation and high Coulombic efficiency.



INTRODUCTION

Recently, bioethanol has become a popular alternative fuel, and its worldwide production from agricultural materials and cellulosic biomass has grown extensively.[1] In 2010, world ethanol production reached 84 billion liters (22 billion gallons), which was about 5 times the production at the start of decade (*F.O. Licht, World Ethanol and Biofuels 2011*). However, a key problem is that bioethanol manufacturing generates large volumes of high-strength wastewater, mainly from the distillation process;[2−4] on average, 8−15 L of distillery wastewater (DWW) are generated for each liter of ethanol produced.

DWW is characterized by its extremely high chemical oxygen demand (COD), typically 80−100 g/L, and also its dark brown color and a high sulfate concentration (1.3−3.7 g/L).[2,3] The pollution potential of wastewater from a medium-sized ethanol distillery is equivalent to the sewage of a city with a population of 500,000.[2] Obviously, adequate treatment is required before DWW can be discharged into the environment.[5] However, the high COD content also offers the potential for energy recovery.[6]

Due to the high temperature inherent in the distillation process, the temperature of the wastewater stream also is high, typically from 70 to 80 °C[4]. Since the high temperature may require cooling to satisfy mesophilic conditions, it would be beneficial to perform thermophilic treatment of DWW in conjunction with an energy-recovery process.

Commonly, DWW is treated by anaerobic digestion (AD) to generate methane fuel. However, the high level of sulfate in DWW can inhibit the methanogenic activity. In addition, the generation of sulfide from sulfate reduction might contribute to bad odor in the surrounding environment and corrosion in the treatment system.[2]

Microbial fuel cells (MFCs) provide a promising technology for attaining a sustainable energy source, while concurrently oxidizing organic pollutants[7−9] and sulfide[10] in wastewater. Recently, molasses distillery wastewater was examined as an organic fuel for electricity production in a mesophilic MFC.[5,11] However, studies on thermophilic MFCs are few,[12,13] and none are directed toward the treatment of wastewater. Nevertheless, the thermophilic operation of MFCs may produce benefits by

Received: October 30, 2011
Revised: January 14, 2012
Accepted: January 26, 2012
Published: January 26, 2012

reducing the activation resistance, mass-transfer limitation, and ohmic potential loss; indeed, thermophilic MFCs have shown improved rates of electron production in MFC operations.[14,15]

In contrast with the broad knowledge about mesophilic MFCs, current information about microorganisms that have the capacity for extracellular electron transfer to a solid electrode in a thermophilic MFC is minimal. These microbes are called electrochemically active bacteria (EAB)[7] or anode-respiring bacteria (ARB) due to their unique ability to respire via electron transfer to the anode. Previous community analyses from thermophilic MFCs focused on systems using acetate as the sole electron donor.[12,13,16] Studies analyzing 16S rRNA revealed that the predominant clones were mostly related to Gram-positive thermophiles in the Firmicutes and Deferribacteres.[12,13] Wrighton et al.[16] combined 16S rDNA abundance and 16S rRNA expression measurements using a PhyloChip to show that Firmicutes were dominant and probably involved in electricity generation in their thermophilic MFC anode. It is not known if Gram-positive Firmicutes and/or Deferribacteres remain dominant in thermophilic MFCs used for treating wastewater of complex organic substrates.

The overarching goals of this study are (1) to evaluate whether or not thermophilic MFCs can achieve high efficiency for electricity production and also can remove sulfate along with oxidizing organic substrates in this high-temperature wastewater, and (2) to explore the bacterial community structure in the anode of thermophilic MFCs in response to MFC performance. To achieve these goals, we measured the energy-conversion efficiency and removal efficiencies of COD and sulfate during treatment of DWW by thermophilic MFCs. We explored the bacterial diversity in a thermophilic anode biofilm, along with the inoculum, using pyrosequencing of the bacterial 16S rRNA gene amplicon, which gave reads long enough (~400 bp) to identify bacteria at the species level.[17]

EXPERIMENTAL SECTION

MFC Construction and Operation. Plate-type mediatorless MFCs[18] were used in this study. The anode and cathode compartments (20-mL each) were separated by a Nafion 424 cation exchange membrane (DuPont, Wilmington, DE, USA) and contained two sheets (12 mm × 2 mm × 0.8 mm) of graphite felt (Electrosynthesis Co.,Lancaster, NY, USA) as electrodes. The external resistance was 10 Ω, and peristaltic pumps (Watson-Marlow, Falmouth, Cornwall, UK) were used to feed both compartments of the MFCs at desired flow rates. The anode compartment received DWW (described below) fed via an up-flow pump-delivery system; concurrently, the cathode compartment was continuously fed with a phosphate buffer (pH 7.0; 50 mM) at the constant feeding rate of 20 mL/min. All MFCs were installed in a temperature-controlled chamber (55 °C).

Thermophilic anaerobic digestion sludge (55 °C) collected from the wastewater treatment process of Jinro Distillers Co. (Ansan, Korea) was used as the inoculum, and fresh effluent wastewater discharged from the distillery process was collected at a high temperature (around 80 °C) for use as the DWW feed. Due to constraints imposed by the MFC configuration (a small anode chamber for up-flow continuous feeding), only the liquid phase of the wastewater could be applied. Thus, the wastewater was centrifuged (6000 rpm, 20 min) to separate the liquid and solid phases; both were stored in a deep freezer (−70 °C) prior to use in further experiments. To eliminate nonbacterial factors that might limit performance, the high-strength DWW was diluted with phosphate buffer to a strength workable with the laboratory-scale MFC (150−1000 mg COD/L).

After inoculation, microbes in the anode chambers were starved for 24 h before continuous feeding with the DWW feed medium. The feed medium was prepared by dissolving inorganic salts and trace minerals into a phosphate buffer (pH 7.0, 50 mM), as described by Chang et al.[18] The medium was then autoclaved at 121 °C (15 min) before being cooled under nitrogen gas atmosphere. When the medium was cooled to around 40 °C, the liquid phase of the DWW was added into the medium bottle to the desired COD value, and the medium was gassed with N_2 before supplying it to the MFCs. During operation, a gastight bag (SKC, Eighty Four, PA, USA) containing oxygen-free nitrogen was connected in order to maintain the desired anaerobic conditions in the medium bottle. The same operating conditions and inoculum were subsequently applied to operate thermophilic MFCs fed solely with acetate as fuel.

At first, all MFCs were enriched and operated with bare cathode electrodes. To identify the maximum capability of system, the two sheets of the cathode electrode were replaced by Pt-coated graphite felts to minimize the cathode limitation. Pt powder had been sprayed on one side of the graphite felt at a density of 0.3 mg/cm^2.

Process Monitoring. The potential between the anode and the cathode was measured using a digital multimeter (Keithley 2700, Keithley Co., Cleveland, OH, USA) and recorded on a personal computer through a data acquisition system (EXCELINK, Keithley Co.) at 5-min intervals. The measured potential was then converted to current according to Ohm's law [potential (V) = current (I) × resistance (R)]. In addition, the current was converted to coulombs (C) using the equation [Current (A) = Coulomb (C)/Time (s)], and the Coulombic Efficiency (CE) was calculated by integrating the measured current and the maximum current possible based on the observed COD removal. For continuously operated systems, the CE was calculated as[8]

$$\mathrm{CE} = \frac{MI}{Fbq\Delta\mathrm{COD}}$$

where M = 32 g/mol O_2, F is Faraday's constant (96,500 C/mol e$^-$), b = 4 is the number of electrons exchanged per mole of oxygen, q is the volumetric influent flow rate, and ΔCOD is the difference in the influent and effluent COD. We also normalized the current and power densities to the surface area of the anode electrodes.

COD was measured using a COD kit (Humas Co., Daejeon, Korea), and sulfate levels were determined using ion chromatography using AS14 column for anion detection (Dionex Co. Sunnyvale, CA, USA). A solution of Na_2CO_3 and $NaHCO_3$ (2 mM:2 mM) was used as the eluent at a flow rate of 0.95 mL/min. Soluble sulfide in the effluent was determined via the methylene blue method using a kit (Humas Co.).

DNA Extraction, 16S rRNA Gene Amplification, and FLX Titanium Pyrosequencing. The graphite felt electrodes from the anode compartments of the thermophilic MFCs were sampled for DNA extraction after 1 year of operation. Metagenomic DNA was extracted using the PowerSoil DNA isolation kit (MOBIO, Carlsbad, CA, USA) according to the manufacturer's instructions. For comparison, metagenomic DNA also was extracted from the graphite felt anodes of an

acetate-fed thermophilic MFC that was operated in the same way and from the initial inoculum of activated sludge. Three samples were taken from each source.

Fragments of 16S rRNA genes within the variable V1−V3 region were amplified from the extracted DNA using primer sets 27F (GAGTTTGATCMTGGCTCAG) and 518R (WTTACCGCGGCTGCTGG). The 9 different bar codes (GACACTGT, GAGTACAG, and GCTATAGC for Ace_MFC sample (acetate fed MFC), GTAGCATC, GTCACAGT, and TAGCGCAT for DWW_MFC sample (DWW fed MFC), and TATAGCGC, TCGAGTAC, and TGAGTCTG for the inoculum sample) were used to sort each sample in the pyrosequencing runs, in which sample sequences were mixed. Each PCR reaction was performed as described by Lee et al.[19] After PCR amplification, the amplicons were purified once by gel electrophoresis and then purified twice using a QIAquick gel extraction kit (Qiagen, Valencia, CA, USA) and a QIAquick PCR purification kit (Qiagen). Pyrosequencing of the amplicons was performed by Macrogen Inc. (Seoul, Korea) using a 454/Roche GS-FLX Titanium instrument (Roche, Branchburg, NJ, USA). Finally, low-quality sequences were filtered out according to previous studies[20,21] using the cutoff values for read length (<300 nucleotides), the number of ambiguous sequences (>0), and average quality score (QS < 20).

Microbial Community Analysis. Multiple sequence alignments and complete linkage clusterings were used to cluster sequences from 0 to 10% dissimilarity using the RDP pyrosequencing pipeline.[22] These clusters served as operational taxonomic units (OTUs) for generating rarefaction curves and for calculating OTU richness and diversity indexes. Representative sequences from each OTU were classified to match closest strains by RDP Classfier,[22,23] EzTaxon,[24] Greengenes,[25] and BLASTN.[26] To construct a phylogenetic tree based on the 16S rRNA genes, the pyrosequenced sequences were aligned with known reference sequences using MUSCLE,[27] and then a phylogenetic tree was produced using MEGA4[28] by using a neighbor-joining algorithm employing a similarity matrix of pairwise comparisons with 1000 bootstrap replicates.

RESULTS

Enrichment and Electricity Production from Thermophilic MFC Operated with DWW. The MFC anodes were fed continuously with 300 ± 17 mg COD/L DWW at a rate of 0.45 mL/min (equivalent to 9.7 ± 0.6 kg COD/m^3day). Figure 1 presents the current and power developed from a thermophilic MFC operated with DWW. An open-circuit potential of about 0.65 ± 0.02 V developed in all MFCs to which thermophilic anaerobic digester sludge was added. When the electrodes were connected through an external resistance of 10 Ω, the potential dropped immediately to 0.01 mV before starting to gradually increase. After 2 weeks of operation, the current reached 5 ± 0.3 mA (0.71 ± 0.04 A/m^2 and 250 ± 14 A/m^3) (Figure 1A). The COD removal was 66.5 ± 2.8%, and the CE was 27.3 ± 1.1%.

When the two cathode sheets were sequentially replaced by Pt-coated graphite felt, the current production increased rapidly. The current increased to 11.5 ± 0.3 mA (1.6 ± 0.04 A/m^2 and 600 ± 15 A/m^3) and 16.5 ± 0.2 mA (2.4 ± 0.03 A/m^2 and 838 ± 10 A/m^3), respectively, with one and two sheets of Pt-coated electrode (Figure 1A). In parallel, the CE increased from 27.3% to 72.4% and 89.3%, respectively.

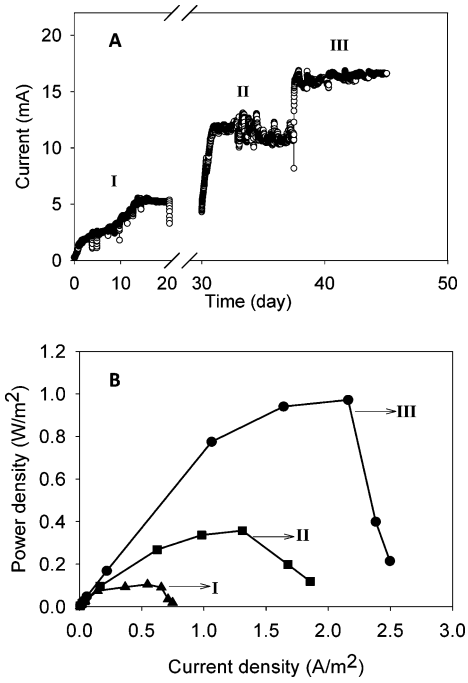


Figure 1. Current (A) and power (B) production from thermophilic MFC operated with DWW at different cathode electrode conditions (bare (I), one Pt-coated sheet (II), and two Pt-coated sheets (III)).

The maximum power density, obtained from polarization curves obtained by varying the external resistance (5 Ω to 40 kΩ), was only about 0.1 ± 0.003 W/m^2 (37 ± 1 W/m^3) with the bare cathode MFC, but significantly increased to 0.36 ± 0.01 W/m^2 and 0.97 ± 0.02 W/m^2 (126 ± 4 W/m^3 and 342 ± 7 W/m^3, respectively) in MFCs that had one and two sheets of Pt-coated cathode electrode, respectively (Figure 1B).

Bioelectrochemical Treatment of DWW. *COD Removal and CE.* The COD removal efficiency of the thermophilic MFC varied with the strength of the DWW. Figure 2 shows the relationships among DWW strength, current generation, COD removal, and CE for thermophilic MFCs having one Pt-coated cathode. The DWW strength was diluted to a range of 142 ± 7 to 1043 ± 20 mg/L (loading rate range from 4.6 ± 2.0 to 33.8 ± 0.7 kg COD/m^3day). In general, increasing the DWW strength led to a higher current density (from 1.1 ± 0.04 to 2.0 ± 0.1 A/m^2) for the same influent flow rate. However, the increase was small for an influent COD above 300 mg/L. In contrast, COD removal efficiency declined with a higher influent COD (from 76 ± 3% to 46 ± 2%), although COD removal stabilized at 46% for an influent COD over 700 mg/L. Consequently, the CE decreased with DWW strength: 81 ± 2% for 142 ± 7 mg/L COD, declining to 31.5 ± 2% at 1043 ± 20 mg/L (Figure 2B). The plot of the current production at each wastewater strength, shown as dotted lines in Figure 2A, indicates that the maximum current density with the thermophilic MFC was about 2.2 ± 0.2 A/m^2, and that the apparent half-maximum rate concentration (K_s) of the thermophilic anode respiring bacteria with DWW was 117 ± 47 mg COD/L (R^2 = 0.90). Regardless of the acetate concentration fed to the anode, the thermophilic acetate-fed MFCs recorded a CE value of more than 95%.

Sulfate Reduction during MFC Operation. The reduction of sulfate was examined in our thermophilic MFCs. The sulfate concentration varied with the strength of wastewater fed to the

dx.doi.org/10.1021/es203861v | Environ. Sci. Technol. 2012, 46, 3022−3030

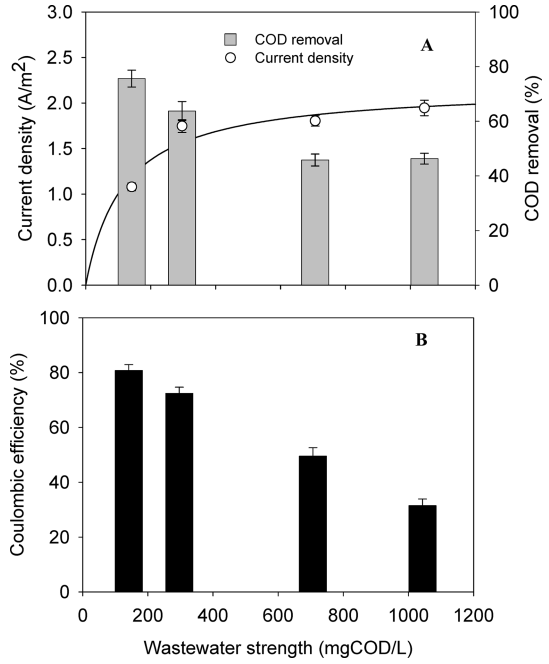


Figure 2. Relationship between initial DWW strength with COD removal and produced current density (A) and CE (B) in the thermophilic MFC with a cathode of one Pt-coated sheet.

anode, ranging from 14.0 ± 1.3 to 42.1 ± 3.7 mg/L. Regardless of the DWW strength, a sulfate removal efficiency of around 60% was obtained in all experiments, as shown in Table 1.

It is known that sulfate reduction in anaerobic treatments results in the formation of sulfide that may appear in the effluent.[29] Here, we observed that the sulfide concentration in the effluent during treatment of sulfate-containing DWW was significantly less than theoretically produced sulfide from sulfate removal (Table 1). This removed sulfide could be oxidized via the generation of electricity in MFCs[10]

Bacterial Populations in Thermophilic MFCs and Inoculum. Approximately 80% of the raw reads passed the quality-filtering standards, and this gave 7883 ± 3426, 13 438 ± 3756, and 9472 ± 3544 filtered reads, respectively, for the DWW-MFC, Ace-MFC, and inoculum; the average lengths of the filtered sequence reads were 481 ± 34 bps, 463 ± 36 bps, and 437 ± 33 bps for the same samples. The numbers and lengths of the quality-filtered sequence reads are within typical ranges reported in previous reports.[19]

In the inoculum, more than 70% of the bacterial members were classified into the Thermotogae class (Figure 3A). The second and third major groups belong to the Clostridia (17%) and Synergistia (4%) classes; other classes were diverse and formed very small fractions of the total bacteria population. On the anode of the DWW-fed MFC, however, Bacteroidia, Nitrospira, and Delta-proteobacteria were selectively enriched compared to the inoculum (Figure 3B). The major bacterial members belonged to the classes of Bacterioidia (52% of total bacterial community), Clostridia (20%) unclassified class (11%), Nitrospira (6.1%), Beta-proteobacteria (4%), Delta-proteobacteria (3%), and Synergistia (2.5%). On the anode of acetate-fed MFC, the major enriched bacterial members were classified into Deferribacteres (26%) and Sphingobacteria (19%), neither of which was important in the DWW-fed MFC or inoculum (Figure 3C).

DWW-fed and acetate-fed thermophilic MFCs stimulated populations in the Beta-proteobacteria class, but suppressed the Thermotogae class. The predominant Bacteroidia classes in the DWW-MFC were neither enriched in the acetate-fed MFC nor important in the inoculum, indicating that the Bacteroidia group was uniquely selected in the DWW-fed MFC.

The Clostridia class members appeared in all samples as large percentages (17−29%). However, Clostridia in the Ace-MFC were mainly composed of the *Coprothermobacter* genus (96.3% of total Clostridia and 28.0% of total bacterial population), whereas the Clostridia members in the DWW-MFC sample were diverse.

Identified Dominant Bacteria in the DWW-Fed Thermophilic MFC. 16S rRNA gene sequences for the DWW-MFC sample were clustered into OTUs with a sequence similarity of >97% (equivalent to species level[30]); the dominant bacterial OTUs are listed in Table 2. With one exception (D3), the other dominant bacterial OTUs in the table were present only in the DWW-MFC. The most dominant OTU population (D1; with 39.1% of the total bacterial community) is a close relative (97.8% identity) to an uncultured *Bacteriodetes* bacterium, uniquely present in an anaerobic digester, from among the seven tested for treating municipal wastewater treatment sludge.[31] The D3 OTU commonly detected from the three samples is closely related (100% identity) to uncultured *Coprothermobacteria* bacterium clone from one of the anaerobic digesters studied by Riviere et al.[31] The D5 OTU is close to *Thermodesulfovibrio aggregan* TGE-P1, a known thermophilic sulfate reducer isolated from methanogenic anaerobic sludge.[32] The D6 OTU was close (99.5% identity) to an uncultured *Bacillus* bacterium clone obtained from a thermophilic anaerobic consortium using propionate.[33] The D2, D4, D7, and D8 OTUs were not identifiable because of their identities lower than 97%.

DISCUSSION

Complex organic compounds contained in DWW were effectively degraded, with most of the electron equivalents converted to electricity in the thermophilic MFC system. When

Table 1. Reduction of Sulfate Concentration at the Different Wastewater Strengths and Potential Current Production from Sulfide Oxidation

	sulfate concentration (mg/L)			sulfide concentration (mg/L)		
feeding condition (mg COD/L)	influent	effluent	sulfate removal (%)	theoretically produced from sulfate reduction[a]	measured sulfide in effluent	potential current from sulfide oxidation (mA)[b]
296.65 ± 10.1	14.0 ± 1.3	5.7 ± 0.9	59.3 ± 3.2	2.85 ± 0.30	0.34 ± 0.07	0.11
706.75 ± 9.5	28.5 ± 3.5	11.2 ± 3.1	60.7 ± 1.3	5.94 ± 0.20	0.95 ± 0.07	0.21
1043.35 ± 20.3	42.1 ± 3.7	18.0 ± 3.4	57.2 ± 0.8	8.28 ± 0.20	2.21 ± 0.04	0.26

[a]Theoretical sulfide concentration was calculated from sulfate removal. [b]Potential current was calculated by oxidation of sulfide to sulfur.

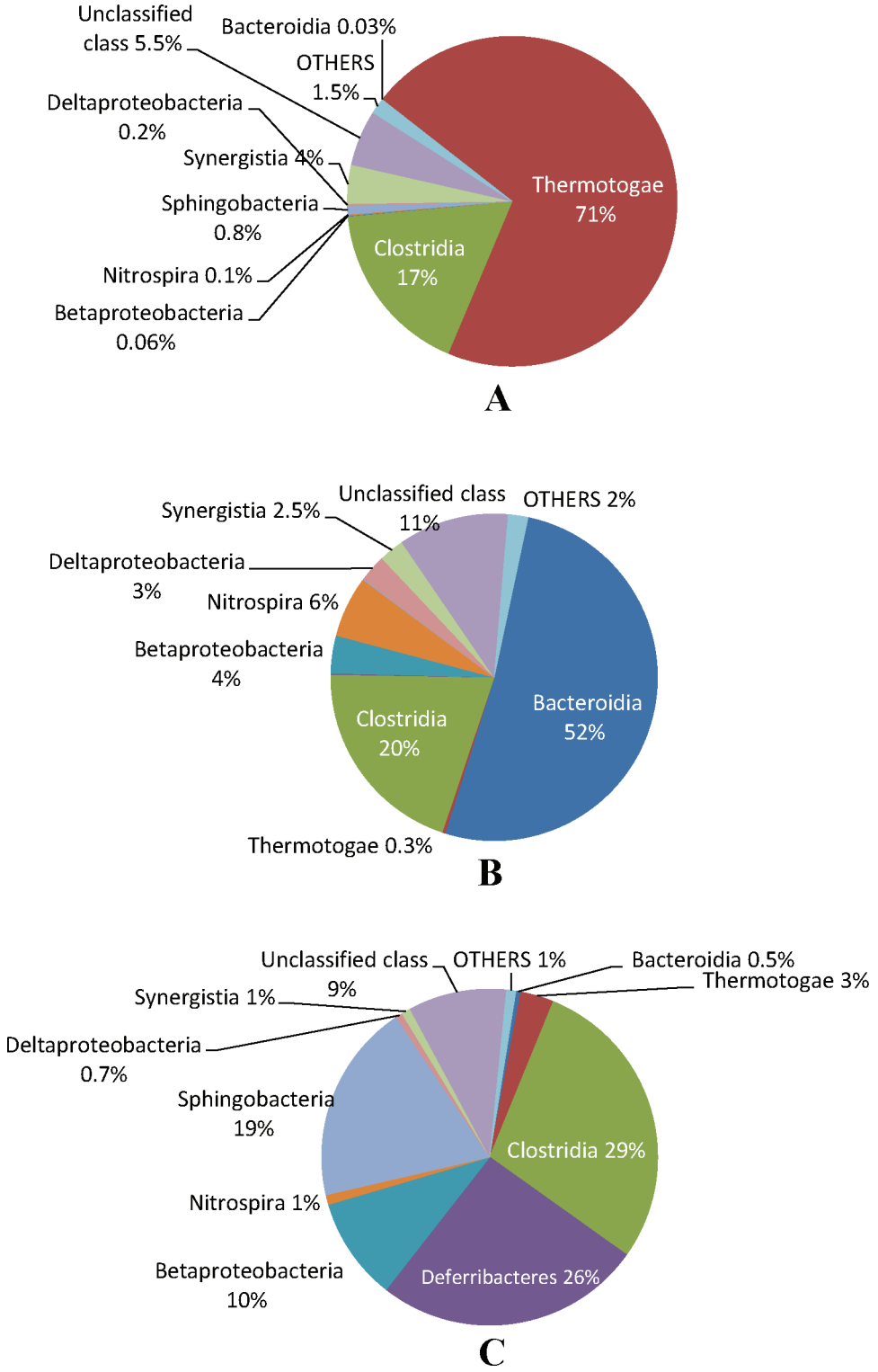


Figure 3. Bacterial community structure changes in response to thermophilic MFC treatments from anaerobic digested initial inoculums (A) to DWW-fed MFC (B) and acetate-fed MFC (C).

Pt was employed on the cathode electrode, the catalytic activity of cathode was enhanced. This switched the electron distribution in favor of the anode of DWW-fed MFC: from only 27% as electrical current to over 89%. Thus, the higher CE value (over 89%) reflects that the electrical production by ARBs was favored over other electron acceptors, such as sulfate reducing and methanogenesis, when the cathode reaction was made more efficient. To our knowledge, this CE value is the highest efficiency value obtained from an MFC operated with wastewater. This result also is comparable with previous operations of thermophilic MFCs fed acetate as the sole electron donor.[12,16]

The maximum current and power density produced by a single MFC cell in this study were 2.4 A/m^2 (838 ± 10 A/m^3)

dx.doi.org/10.1021/es203861v | *Environ. Sci. Technol.* 2012, 46, 3022–3030

Table 2. Dominant Bacterial Populations from DWW for Thermophilic MFC Anode Biofilm Community

OTU ID	RA[a] (%)	accession no.	closest match with Greengenes whole database	identity (%)	accession no.	source
D1	39.1	JN851061	uncultured Bacteroidetes bacterium clone	97.8	CU924484	28
D2	7.6	JN851062	uncultured bacterium clone from a hot spring	89.9	FM994916	unpublished
D3	3.4	JN851063	uncultured *Coprothermobacteria* bacterium clone	99.8	CU924707	28
D4	1.9	JN851064	*Aminomonas paucivorans* DSM 12260, a mesophilic, anaerobic, amino-acid-utilizer	85.8	AF072581	44
D5	1.7	JN851065	*Thermodesulfovibrio aggregan* TGE-P1, a sulfate reducer in thermophilic methanogenic sludge	99.5	AB021302	29
D6	1.2	JN851066	uncultured *Bacillus* clone, from a thermophilic anaerobic consortium using propionate	99.5	AB332117	30
D7	1.1	JN851067	uncultured *Firmicutes* bacterium clone	91.5	CU925643	28
D8	1.1	JN851068	*Bacteroides* sp. SA-11, an isolate degrading lindane and cellulose	94.9	AY695842	unpublished

[a]RA indicates relative abundance.

and almost 1.0 W/m^2 (342 ± 7 W/m^3), respectively, which are comparable to or higher than reported previously from wastewater-treating MFCs.[34−37] In the reviews by Kim et al.[38] and Logan,[39] the highest electricity outputs, several amperes/watts per square meter of anode surface, were only achieved with MFC systems operated with a sole substrate, such as acetate;[40,41] in some cases it was using ferricyanide at the cathode.[42] Recently, DWW also was applied to an air-cathode MFC under mesophilic conditions,[11] and the maximum current and power output were only 0.35 A/m^2 and 124 mW/m^2, respectively. Thus, our results suggest an advantage of DWW treatment with thermophilic MFCs.

Increases in wastewater strength lowered the efficiency of electricity production in our thermophilic MFCs (Figure 2B). This rate-saturation effect was due to the fixed surface area of the anode, since the activity of ARBs depends on diffusion and electron-conduction processes that depend on surface area.[43]

The high sulfate level of DWW is noteworthy, and we saw about 60% sulfate reduction in our thermophilic MFCs. This reduction of sulfate in our DWW-fed MFC was caused by thermophilic sulfate reducers, which is supported by pyrosequencing results showing the enrichment of a D5 species that is phylogenetically close to *Thermodesulfovibrio aggregan* TGE-P1, a known sulfate-reducing thermophile found in AD processes.[32]

The decreasing amount of sulfide produced in the effluent during operation indicated that sulfide was oxidized in anode. Sulfide oxidation in the MFC could donate electrons to the electrode to produce an electrical current.[10] However, within the range of DWW strength used in this study, the current obtained from sulfide oxidation was almost negligible compared with observed current (Table 1). Thus, the results from this study confirmed that thermophilic MFCs, which require less energy for cooling the DWW, can achieve high efficiency for electricity production and also can reduce sulfate along with oxidizing organic substrates in this high temperature wastewater.

The pyrosequencing profile of the bacterial communities from the anode electrode of the DWW-fed MFC show a different community structure compared to those from the inoculum and the acetate-fed MFC. Whereas members of the Thermotogae class appeared to have important roles in the thermophilic anaerobic digester, they were inhibited or outcompeted in the thermophilic MFC systems. Similar to previous findings,[12,13,16] the members of Gram-positive Clostridia (Firmicutes phylum) and Deferribacteres were selected as the dominant communities in anode of thermophilic acetate-fed MFC. In contrast, the thermophilic DWW-fed MFC had a predominance of Gram-negative Bacteroidia (Bacteroidetes phylum), up to 52% of the total bacterial population. Although members of Bacteroidia class have been reported to be dominant in some mesophilic MFCs,[44,45] this is the first observation that Gram-negative Bacteroidia thermophiles emerge as the strong competitor in thermophilic MFC fed with complex DWW.

The difference in bacterial community composition between the inoculum and the DWW-fed MFC probably resulted more from anode-respiration rather than high temperature and wastewater complexity, since the temperature and wastewater conditions were similar. The difference in bacterial community composition between the acetate-fed and the DWW-fed MFCs may have resulted mainly from an effect by difference in wastewater complexity, since the both thermophilic MFCs were operated in similar temperature and MFC reactor conditions. The high CE values for the thermophilic MFCs (up to 89% for DWW-fed MFC and 95% for acetate-fed MFC) suggested that ARB may have been selectively enriched from the inoculum during the MFC operations.

With the thermophilic DWW-fed MFC, one uncharacterized *Bacteroidetes* clone (OTU D1) dominated up to 39% of total reads. The co-occurrence of the selection of dominant bacterium with high electricity generation and high Coulombic efficiency in this study suggest that this novel *Bacteroidetes* species had an important role related to electricity generation from the DWW. Several *Bacteroidetes* species have been reported for their electrochemical activities in mesophilic MFCs.[44,45] However, due to the lack of available information on anode-respiring thermophiles, it remains unclear what conditions in the anode stimulated the predominance of this D1 bacterium in thermophilic MFC. Further isolation and characterization will be needed to provide deeper insight about its function.

An additional dominant OTU D3 was detected commonly in all three samples (D3 = A2 = I13) (Supporting Information Supplementary Tables 1 and 2) and was closely related (100% identity) to an uncultured *Coprothermobacteria* bacterium clone from one of the anaerobic digesters studied by Riviere et al.[31] The *Coprothermobacter* genus has been found to be dominant in previous high-efficiency thermophilic acetate-fed MFCs.[12−16] In addition, the significant numbers of this bacterium in the anode of the thermophilic acetate-fed MFC in our study (up to 12% of total population; Supplementary Table 1) might suggest that this D3 bacterium was a thermophilic acetate-ultilizing ARB. Thus, this bacterium have been selectively enriched based on its ability to carry out current production in DWW-fed MFC and acetate-fed MFC.

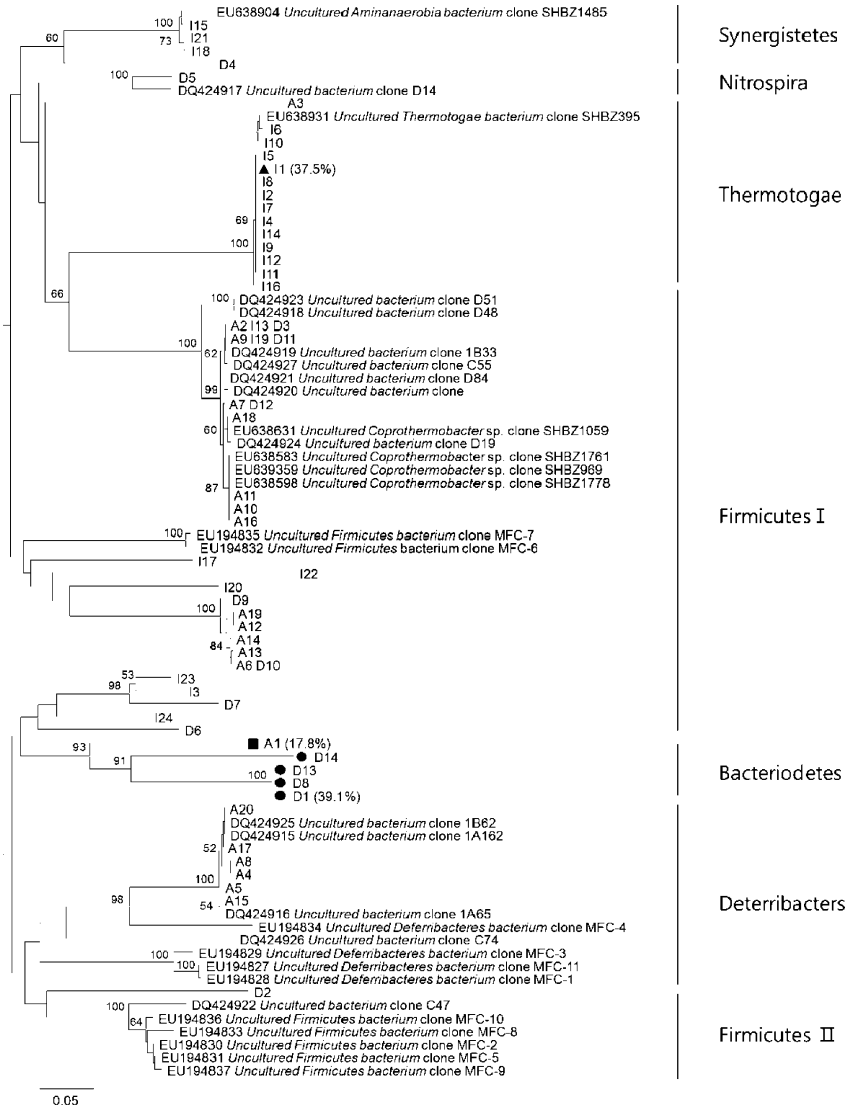


Figure 4. Phylogenetic tree constructed showing dominant OTUs from DWW-fed thermophilic MFC (D), acetate-fed thermophilic MFC (A), and anaerobically digested initial inoculum (I). Relative abundances of the most-dominant OTUs (A1, D1, I1) are shown in parentheses. Sequences that had been previously identified from thermophilic MFC studies[12,13,16] are used as reference sequences. The scale bar represents 0.05 substitution per nucleotide position.

The significantly high densities of possible ARB, represented by uncultured *Bacteroidetes* bacterium D1 and uncultured *Gamma-proteobacteria* bacterium D3 (39.1% and 3.4% total population, respectively), compared with that of SRB, represented by *Thermodesulfovibrio aggregan* D5 (1.7%), in the anode of DWW-fed MFC agrees with the observation that electrical current was the major electron sink. In the thermophilic acetate-fed MFC, however, the high current production and CE might have come from the relatively high abundances of *Coprothermobacter* (96% of Clostridia and 28% of total bacterial population) and Deferribacteres (25% of total bacterial population). Both have been suggested to have ability to do extracellular electron transfer in previous thermophilic acetate-fed MFC studies.[12,13,16]

While most of the dominant bacterial OTUs from the acetate-fed MFC and the inoculum are close to known bacterial species (Supplementary Tables 1 and 2), some of the dominant bacterial OTUs from the DWW-fed MFC are distant from known bacteria species, with a 16S rRNA sequence similarity lower than 96%. A phylogenetic tree (Figure 4) was constructed for the representative 16S rRNA gene sequences of each OTU, together with reference sequences, based on previously reported thermophilic MFC bacterial 16S rRNA gene sequences.[12,13,16] According to the tree, most dominant bacterial species populations from the DWW-fed MFC (such as D1, D8, D13, and D14) are phylogenetically distant from previously known thermophilic MFC bacteria; this contrasts with the acetate-fed MFC and the inoculum, which had many bacteria close to the known thermophilic MFC bacteria (A and I series). These results unveil a high novelty of bacterial populations enriched by a thermophilic MFC fed with high-complex DWW.

■ ASSOCIATED CONTENT

Supporting Information

Supplementary Tables 1 and 2 and references. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: ischang@gist.ac.kr; tel: +82-62-715-3278; fax: +82-62-715-2434.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by a grant from the *Doyak* Program (previously the NRL Program, R0A-2008-000-20088-0) and the World Class University (WCU) program (R33-10076) of the National Research Foundation of Korea, funded by the Ministry of Education, Science and Technology.

REFERENCES

(1) Pant, D.; Adholeya, A. Biological approaches for treatment of distillery wastewater: A review. *Bioresour. Technol.* **2007**, *98* (12), 2321−2334.

(2) Wilkie, A. C.; Riedesel, K. J.; Owens, J. M. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. *Biomass Bioenergy* **2000**, *19* (2), 63−102.

(3) Satyawali, Y.; Balakrishnan, M. Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: A review. *J. Environ. Manage.* **2008**, *86* (3), 481−497.

(4) Mohana, S.; Acharya, B. K.; Madamwar, D. Distillery spent wash: Treatment technologies and potential applications. *J. Hazard. Mater.* **2009**, *163* (1), 12−25.

(5) Zhang, B.; Zhao, H.; Zhou, S.; Shi, C.; Wang, C.; Ni, J. A novel UASB−MFC−BAF integrated system for high strength molasses wastewater treatment and bioelectricity generation. *Bioresour. Technol.* **2009**, *23*, 5687−5693.

(6) Rittmann, B. E. Opportunities for renewable bioenergy using microorganisms. *Biotechnol. Bioeng.* **2008**, *100* (2), 203−212.

(7) Chang, I. S.; Moon, H.; Bretschger, O.; Jang, J. K.; Park, H. I.; Nealson, K. H.; Kim, B. H. Electrochemically active bacteria and mediator-less microbial fuel cell. *J. Microbiol. Biotechnol.* **2006**, *16*, 163−177.

(8) Logan, B. E.; Hamelers, B.; Rozendal, R.; Schrőder, U.; Keller, J.; Frequia, S.; Aelterman, P.; Verstraete, W.; Rabaey, K. Microbial fuel cells: Methodology and technology. *Environ. Sci. Technol.* **2006**, *40*, 5181−5192.

(9) Rabaey, K.; Ossieur, W.; Verhaege, M.; Verstraete, W. Continuous microbial fuel cells convert carbohydrates to electricity. *Water Sci. Technol.* **2005**, *52* (1−2), 515−523.

(10) Rabaey, K.; Van De Sompel, K.; Maignien, L.; Boon, N.; Aelterman, P.; Clauwaert, P.; De Schamphelaire, L.; Pham, T. H.; Vermeulen, J.; Verhaege, M.; Lens, P.; Verstraete, W. Microbial fuel cells for sulfide removal. *Environ. Sci. Technol.* **2006**, *40*, 5218−5224.

(11) Mohanakrishna, G.; Venkata Mohan, S.; Sarma, P. N. Bio-electrochemical treatment of distillery wastewater in microbial fuel cell facilitating decolorization and desalination along with power generation. *J. Hazard. Mater.* **2009**, *177* (1−3), 487−494.

(12) Jong, B. C.; Kim, B. H.; Chang, I. S.; Pauline, W. Y. L.; Choo, Y. F.; Kang, G. S. Enrichment, performance and microbial diversity of a thermophilic mediator-less microbial fuel cell. *Environ. Sci. Technol.* **2006**, *40*, 6449−6454.

(13) Mathis, B. J.; Marshall, C. W.; Milliken, C. E.; Makkar, R. S.; Creager, S. E.; May, H. D. Electricity generation by thermophilic microorganisms from marine sediment. *Appl. Microbiol. Biotechnol.* **2008**, *78* (1), 147−155.

(14) Du, Z.; Li, H.; Gu, T. A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. *Biotechnol. Adv.* **2007**, *25* (5), 464−482.

(15) Rismani-Yazdi, H.; Carver, S. M.; Christy, A. D.; Tuovinen, O. H. Cathodic Limitations in Microbial Fuel Cells: An Overview. *J. Power Sources* **2008**, *180* (2), 683−694.

(16) Wrighton, K. C.; Agbo, P.; Warnecke, F.; Weber, K. A.; Brodie, E. L.; DeSantis, T. Z.; Hugenholtz, P.; Andersen, G. L.; Coates, J. D. A novel ecological role of the Firmicutes identified in thermophilic microbial fuel cells. *ISME J.* **2008**, *2* (11), 1146−1156.

(17) Wolcott, R. D.; Gontcharova, V.; Sun, Y.; Dowd, S. E. Evaluation of the bacterial diversity among and within individual venous leg ulcers using bacterial tag-encoded FLX and Titanium amplicon pyrosequencing and metagenomic approaches. *BMC Microbiol.* **2009**, *9*, 226.

(18) Chang, I. S.; Jang, J. K.; Gil, G. C.; Kim, M.; Kim, H. J.; Cho, B. W.; Kim, B. H. Continuous determination of biochemical oxygen demand using microbial fuel cell type biosensor. *Biosens. Bioelectron.* **2004**, *19*, 607−613.

(19) Lee, T. K.; Doan, T. V.; Yoo, K.; Choi, S; Kim, C.; Park, J. Discovery of commonly existing anode biofilm microbes in two different wastewater treatment MFCs using FLX titanium pyrosequencing. *Appl. Microbiol. Biotechnol.* **2010**, *87* (6), 2335−2343.

(20) Huse, S. M.; Huber, J. A.; Morrison, H. G.; Sogin, M. L.; Welch, D. M. Accuracy and quality of massively parallel DNA pyrosequencing. *Genome Biol.* **2007**, *8* (7), R143.

(21) Brockman, W.; Alvarez, P.; Young, S.; Garber, M.; Giannoukos, G.; Lee, W. L.; Russ, C.; Lander, E. S.; Nusbaum, C.; Jaffe, D. B. Quality scores and SNP detection in sequencing-by-synthesis system. *Genome Res.* **2009**, *18*, 763−770.

(22) Cole, J. R.; Wang, Q.; Cardenas, E.; Fish, J.; Chai, B.; Farris, R. J.; Kulam-Syed- Mohideen, D. M.; McGarrell, D. M.; Marsh, T.; Garrity, G. M.; Tiedje, J. M. The ribosomal database project: Improved alignments and new tools for rRNA analysis. *Nucleic Acids Res.* **2008**, *37*, 141−145.

(23) Wang, Q.; Garrity, G. M.; Tiedje, J. M.; Cole, J. R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **2007**, *73*, 5261−5267.

(24) Johnson, M.; Zaretskaya, I.; Raytselis, Y.; Merezhuk, Y.; McGinnis, S.; Madden, T. L. NCBI BLAST: A better web interface. *Nucleic Acids Res.* **2008**, *36*, 5−9.

(25) Chun, J; Lee, J. H.; Jung, Y; Kim, M; Kim, S; Kim, B. K.; Lim, Y. W. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* **2007**, *57* (Pt 10), 2259−61.

(26) DeSantis, T. Z.; Hugenholtz, P; Larsen, N; Rojas, M; Brodie, E. L.; Keller, K; Huber, T; Dalevi, D; Hu, P; Andersen, G. L. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* **2006**, *72* (7), 5069−72.

(27) Edgar, R. C. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **2004**, *32*, 1732−1797.

(28) Kumar, S.; Nei, M.; Dudley, J.; Tamura, K. MEGA: A biologistcentric software for evolutionary analysis of DNA and protein sequences. *Brief Bioinform.* **2008**, *9*, 299−306.

(29) Rittmann, B. E.; Mc Carty, P. L. *Environmental Biotechnology: Principles and Applications*; McGraw-Hill Book Co.: New York, 2001.

(30) Kunin, V; Engelbrektson, A; Ochman, H; Hugenholtz, P. Wrinkles in the rare biosphere: Pyrosequencing errors can lead to artificial inflation of diversity estimates. *Environ. Microbiol.* **2010**, *12* (1), 118−23.

(31) Rivière, D.; Desvignes, V.; Pelletier, E.; Chaussonnerie, S.; Guermazi, S.; Weissenbach, J.; Li, T.; Camacho, P.; Sghir, A. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. *ISME J.* **2009**, *3*, 700−714.

(32) Sekiguchi, Y.; Muramatsu, M.; Imachi, H.; Narihiro, T.; Ohashi, A.; Harada, H.; Hanada, S.; Kamagata, Y. *Thermodesulfovibrio aggregans* sp. nov. and *Thermodesulfovibrio thiophilus* sp.nov., anaerobic, thermophilic, sulfate-reducing bacteria isolated from thermophilic methanogenic sludge, andemended description of the genus Thermodesulfovibrio. *Int. J. Syst. Evol. Microbiol.* **2008**, *58*, 2541−2548.

(33) Sugihara, T.; Shiratori, H.; Domoto, R.; Miyake, T.; Yaegashi, M.; Ayame, S.; Kataoka, N.; Miya, A.; Beppu, T.; Ueda, K. Unique diversity content of a thermophilic anaerobic microbial consortium

that utilizes propionate in a synthetic medium. *J. Gen. Appl. Microbiol.* **2007**, *53*, 363−369.
(34) Min, B.; Kim, J. R.; Oh, S. E.; Regan, J. M.; Logan, B. E. Electricity generation from animal wastewater using microbial fuel cells. *Water Res.* **2005**, *39* (20), 4961−4968.
(35) Moon, H.; Chang, I. S.; Kim, B. H. Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell. *Bioresour. Technol.* **2006**, *97* (4), 621−27.
(36) Feng, Y.; Wang, X.; Logan, B. E.; Lee, H. Brewery wastewater treatment using air-cathode microbial fuel cells. *Appl. Microbiol. Biotechnol.* **2008**, *78*, 873−880.
(37) Lu, N.; Zhou, S.-G.; Zhuang, L.; Zhnag, J.-T.; Ni, J.-R. Electricity generation from starch processing wastewater using microbial fuel cell technology. *Biochem. Eng. J.* **2009**, *43*, 246−251.
(38) Kim, I. S.; Chae, K. J.; Choi, M. J.; Verstraete, W. Microbial fuel cells: Recent advances, bacterial communities and application beyond electricity generation. *Environ. Eng. Res.* **2008**, *13*, 51−65.
(39) Logan, B. E. Scaling up microbial fuel cells and other bioelectrochemical systems. *Appl. Microbiol. Biotechnol.* **2010**, *85*, 1665−1671.
(40) Logan, B.; Cheng, S.; Watson, V.; Estadt, G. Graphite fiber brush anodes for increased power production in aircathode microbial fuel cells. *Environ. Sci. Technol.* **2007**, *41* (9), 3341−3346.
(41) Fan, Y.; Sharbrough, E.; Liu, H. Quantification of the internal resistance distribution of microbial fuel cells. *Environ. Sci. Technol.* **2008**, *42* (21), 8101−8107.
(42) Rabaey, K.; Lissens, G.; Siciliano, S. D.; Verstraete, W. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. *Biotechnol. Lett.* **2003**, *25* (18), 1531−1535.
(43) Torres, C. I.; Marcus, A. K.; Lee, H. S.; Parameswaran, P.; Brown, R. K.; Rittmann, B. E. A kinetic perspective on extracellular electron transfer by anode-respiring bacteria. *FEMS Microb. Rev.* **2010**, *34*, 3−17.
(44) Kim, G. T.; Webster, G.; Wimpenny, J. W.; Kim, B. H.; Kim, H. J.; Weightman, A. J. Bacterial community structure, compartmentalization and activity in a microbial fuel cell. *J. Appl. Microbiol.* **2006**, *101*, 698−710.
(45) Zhang, Y.; Min, B.; Huang, L.; Angelidaki, I. Generation of electricity and analysis of microbial communities in wheat straw biomass-powered microbial fuel cell. *Appl. Environ. Microbiol.* **2009**, *75* (11), 3389−3395.