Generation of Electricity and Analysis of Microbial Communities in Wheat Straw Biomass-Powered Microbial Fuel Cells[∇]

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Electricity generation from wheat straw hydrolysate and the microbial ecology of electricity-producing microbial communities developed in two-chamber microbial fuel cells (MFCs) were investigated. The power density reached 123 mW/m² with an initial hydrolysate concentration of 1,000 mg chemical oxygen demand (COD)/liter, while coulombic efficiencies ranged from 37.1 to 15.5%, corresponding to the initial hydrolysate concentrations of 250 to 2,000 mg COD/liter. The suspended bacteria found were different from the bacteria immobilized in the biofilm, and they played different roles in electricity generation from the hydrolysate. The bacteria in the biofilm were consortia with sequences similar to those of *Bacteroidetes* (40% of sequences), *Alphaproteobacteria* (20%), *Bacillus* (20%), *Deltaproteobacteria* (10%), and *Gammaproteobacteria* (10%), while the suspended consortia were predominately *Bacillus* (22.2%). The results of this study can contribute to improving understanding of and optimizing electricity generation in microbial fuel cells.

Wheat straw is one of the most abundant renewable resources. According to the Food and Agriculture Organization of the United Nations, approximately 1.9×10^9 tons of wheat straw annually are produced worldwide, accompanied by 6.2×10^8 tons of wheat production. Wheat straw is composed of 35 to 45% cellulose and 20 to 30% hemicelluloses with a relatively low lignin content (<20%) (42). The hemicellulose fraction of the straw is easily hydrolyzed to its constituent sugars by a hydrothermal treatment process, forming a carbohydrate-enriched liquid hydrolysate (46). Chemical and biological approaches to sustainable energy production from the liquefied hydrolysates to energy carriers, such as methane, ethanol, and H_2, have been developed. However, many of these approaches encounter technical and economical hurdles (10, 12, 15, 16). An alternative strategy is direct conversion of wheat straw biomass to electrical energy in microbial fuel cells (MFCs).

MFCs are bioelectrochemical reactors in which microorganisms mediate the direct conversion of chemical energy stored in organic matter or bulk biomass into electrical energy (12, 15, 16, 40). Various substrates, such as simple carbohydrates, low-molecular-weight organic acids, starch, amino acids, chitin, cellulose, domestic wastewater, food-processing wastewater, recycled paper wastewater, and marine sediment organic matter, have been successfully utilized for power generation in MFCs (16–18, 27, 30, 33). To understand the microbial constraints on various fuel-powered MFCs, microbial communities have been characterized by several groups. Microbial communities from various systems are very different and often diverse, ranging from well-known metal- and anode-reducing bacteria

to unknown exoelectrogens (1, 20, 21). It has been found that parameters such as the substrates used as fuels and the inocula used for starting up the MFCs can influence the anode bacterial communities in an MFC, which subsequently influence the efficiency of the MFCs (3, 14, 22, 38, 44). Different pure substrates, such as acetate, glucose, and lactate, were used as fuel to compare the microbial communities that developed in the MFCs. Regardless of the different substrates, all anode communities contained sequences closely affiliated with *Geobacter sulfurreducens* (>99% similarity) and an uncultured bacterium clone belonging to the family *Bacteroidaceae* (99% similarity). *Firmicutes* were only found in glucose-fed MFCs (20). Microbial-community analyses of MFCs powered with complex substrates have also been performed by several researchers, and their results were very diverse. The microbial community in starch wastewater-powered MFC was dominated by unidentified bacteria (35.9%), followed by *Betaproteobacteria* (25.0%), *Alphaproteobacteria* (20.1%), and the *Cytophaga/Flexibacter/Bacteroides* group (19.0%) (21). The anode-attached consortia in a cellulose-powered MFC were related to *Clostridium* spp., while *Comamonas* spp. were abundant in the suspended consortia (13). Although many studies have reported the microbial compositions of MFCs, it is still unclear which microbial communities develop as a function of the external parameters.

Wheat straw biomass constitutes a large source for bioenergy production and shows promising prospects for electricity generation in MFCs. Therefore, wheat straw biomass was used to study the microbial communities that develop during the operation of an MFC in order to better understand the microbial electrochemical roles and potentially improve MFC performance.

The objectives of this study were to (i) test wheat straw hydrolysate as a potential fuel in an MFC for electricity generation and (ii) study the microbial composition and evolution of electricity-producing communities in a two-chamber MFC system. Phylogenetic-diversity analysis of the enriched consor-

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TABLE 1. Composition of wheat straw hydrolysate[a]

Component	Content
pH	4.87 ± 0.1
TS (%)	4.40 ± 0.01
VS (%)	3.40 ± 0.01
Ash content (%)	1 ± 0.01
SS (g/liter)	0.155 ± 0.05
VSS (mg/liter)	0.32 ± 0.1
COD (g/liter)	37.95 ± 1.31
SCOD (g/liter)	32.05 ± 2.22
VFA (g/liter)	0.68 ± 0.14
Ethanol (g/liter)	ND
Total nitrogen (g/liter)	0.20 ± 0.01
Ammonia (g/liter)	0.03 ± 0.01
Protein (g/liter)	1.09 ± 0.03
Lipids (%)	0.24
Furfurals (g/liter)	0.25 ± 0.04
HMF (g/liter)	0.14 ± 0.02
Phenols (g/liter)	0.142
Lignin (g/liter)	ND
Arabinose (g/liter)	1.3
Xylose (g/liter)	9.27
Glucose (g/liter)	2.94

[a] ND, nondetectable; TS, total solids; VS, volatile solids; SS, suspended solid; VSS, volatile suspended solid; SCOD, soluble COD; VFA, volatile fatty acid; HMF, 5-hydroxymethyl furfural.

tia was conducted to verify the presence of hydrolytic and respiratory anaerobes that could couple hydrolysate oxidation with proton reduction in the anode chamber. This is the first report of exploiting microbial communities for direct conversion of wheat straw hydrolysate to electrical energy in an MFC.

MATERIALS AND METHODS

Pretreatment of wheat straw. The wheat straw was pretreated by liquefaction in three steps, as described by Thomsen et al. (45). The pretreatment resulted in a predominately cellulose-containing solid fraction and a liquid fraction, called the hydrolysate. The composition of the hydrolysate is shown in Table 1. Before use, the hydrolysate was diluted to the desired chemical oxygen demand (COD) concentration (250 to 2,000 mg COD/liter, as indicated) using wastewater or a buffered nutrient medium (31). The concentrations of furfurals and phenols therefore ranged from 0.01 mM to 0.12 mM in this study. It was previously reported that furan derivatives and phenolic compounds did not affect electricity generation from glucose even at concentrations of 10 mM (7). The pH of the diluted solutions was adjusted to 7.0 with 0.1 N NaOH.

MFC construction and operation. H-type two-chamber MFCs were constructed as described by Oh et al. (36). The anode and cathode chambers were separated by a proton exchange membrane (diameter, 15 mm; Nafion 117; DuPont Co.), and the total volume and the working volume of each chamber were 300 and 250 ml, respectively. The anode and cathode electrodes (3 by 7 cm; 42 cm^2) were made of Toray carbon paper (TGPH-120; Etek). The distance between the electrodes was approximately 10 cm. Electrical connections and pretreatment of electrodes were as previously described (36).

Wastewater collected from a primary clarifier (Lyngby Wastewater Treatment Plant, Copenhagen, Denmark) was first amended with hydrolysate and then used as the inoculum and fuel in the anode. Following inoculation and stable power generation, the wastewater medium was replaced with a vitamin-amended nutrient medium as described previously (31). The system was considered to be ready for stable electricity production when the maximum voltage output of one batch cycle was reproducible after the reactor was filled with fresh medium at least twice. The medium in the reactor was replaced when the voltage dropped below 50 mV (resistance, 1,000 Ω). The cathode chamber was filled with 50 mM ferricyanide solution [K$_3$Fe(CN)$_6$ in 50 mM phosphate buffer (NaH$_2$PO$_4$ · H$_2$O, 4.22 g liter^{-1}; Na$_2$HPO$_4$ · H$_2$O, 2.75 g liter^{-1}) adjusted to pH 7.0 with 1 N NaOH] as the catholyte. The cathodic solution was continuously stirred at 300 rpm using a magnetic stirring bar to ensure effective mixing.

The initial pH of all solutions was adjusted to 7.0. MFCs were operated at

room temperature (20°C). All electrode transfers and inoculation procedures were conducted in an anaerobic glove box (Coy Scientific Products).

Electrochemical measurements and calculations. The voltage (V) across an external resistor in the MFC circuit was monitored at 30-min intervals using a multimeter connected to a personal computer. The current (I), power (P = IV), and coulombic efficiency (CE) were calculated as previously described, with the power density normalized to the projected surface area of the anode (15).

Chemical analysis. The COD, pH, ammonia, and total solids (volatile solids) of the wheat straw hydrolysate were analyzed by standard methods as described previously (18). Sugar concentrations (Sigma-Aldrich, Germany) were analyzed by high-performance liquid chromatography (Agilent 1100), and volatile organic acids were measured by gas chromatography (Agilent 6890) as previously described (27). Before high-performance liquid chromatography and gas chromatography analysis, samples were first filtered through 0.2-μm Millipore membrane. Hydrogen and methane in the headspace were analyzed using a gas chromatograph (MicroLab, Arhus, Denmark) equipped with a thermal conductivity detector and a stainless steel column packed with Porapak Q (50/80 mesh). Nitrogen was used as the carrier gas.

SEM. Bacteria attached to the electrodes were visualized using a scanning electron microscope (SEM). Electrodes were removed from the chambers, rinsed with a sterile medium, and immersed in 5% formaldehyde overnight to fix the samples. Then, the samples were dehydrated stepwise in a graded series of water/ethanol solutions (25, 50, 70, 85, 95, and 100%) and then dried. The electrode samples were mounted onto copper specimen mounts with contact adhesive. The samples were then sputter coated in a Polaron E-5100 sputter coater using a gold-palladium target and observed in an FEI (Germany) Quanta 200 FEG SEM. The SEM images were captured digitally (47).

Community analysis. The anode electrodes were removed from the MFCs and rinsed with sterile distilled water to remove attached debris at the end of each stage. The attached biofilm was then scraped from the 2-cm^2 carbon anode using a sterilized scalpel, and suspended microorganisms in the anode chamber were also collected by centrifuging 2 ml of the suspended solution at 13,000 × g for 10 min at 4°C. Genomic DNA was extracted directly using the Qiaamp DNA Stool Mini Kit (Qiagen catalog no. 51504) according to the manufacturer's instructions. The extracted DNA was first amplified using the universal primers 27f (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492r (5′-TAC GGY TAC TTG TTA CGA CTT-3′), and then the products were amplified again with the primer set 357f, containing a GC clamp (5′-CGC CCG CCG CGC GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′), and 518r (5′-ATT ACC GCG GCT GCT GG-3′). The PCR amplifications were performed with a thermocycler (Eppendorf) as described by Muyzer et al. (35). The PCR products (25 μl) were separated using 6% (wt/vol) polyacrylamide gels with a denaturant gradient between 40% and 60%. The Dcode Universal Mutation Detection System (Bio-Rad) was used for denaturant gradient gel electrophoresis (DGGE), which was first run in 0.5× Tris-acetate-EDTA buffer at 120 V for 30 min and subsequently at 60 V for 14 h (60°C). After electrophoresis, the gels were stained using SYBR gold (Bio-Medicine) for 40 min and destained in 0.5× Tris-acetate-EDTA buffer (pH 8.0) before the DNA bands were observed with a Gel-Doc image analyzer (Bio-Rad Laboratories). The similarities between lanes from DGGE were analyzed with Quantity One Software (Bio-Rad Laboratories). Bands of interest were cut and excised from the gel and sent for sequencing (MWG, Germany). The sequences were subjected to Basic Local Alignment Search Tool (BLAST) and Ribosomal Database Project analysis. Phylogeny was determined with the Ribosomal Database Project's classifier and Sequmatch.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences have been deposited in the GenBank database and are available under accession numbers FJ222393 to FJ222403.

RESULTS AND DISCUSSION

Voltage generation using wheat straw hydrolysate. Following inoculation and stable power generation (29 mW/m^2) during the period of enrichment (approximately 15 days) with wastewater medium, the anodic solution was replaced with the hydrolysate-modified nutrient medium. In the first transfer, an initial maximum power density of 13.6 mW/m^2 (0.24 V), with a fixed 1,000-Ω resistor, was achieved (Fig. 1). An abiotic control did not generate any electricity (data not shown). After two additional loadings (third transfer), a stable increase of elec-
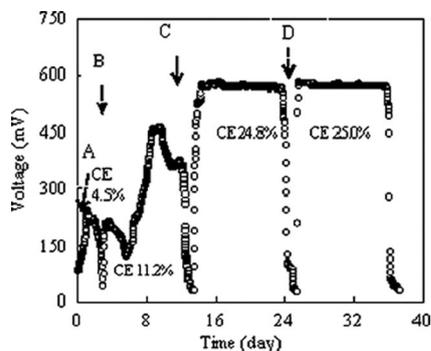
FIG. 1. Voltage output as a function of time from the initial hydrolysate concentration of 1,000 mg COD/liter. A, first transfer; B, second transfer; C, third transfer; D, fourth transfer. The CEs of the transfers (A to D) were 4.5%, 11.2%, 24.8%, and 25.0%, respectively.
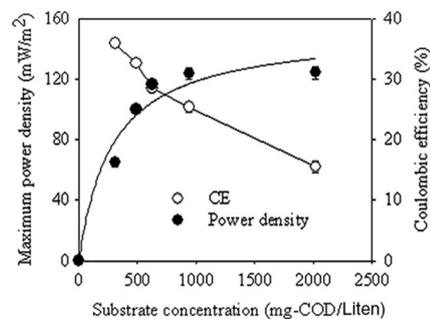

FIG. 2. Maximum power density and CE as functions of the initial substrate concentration (the error bars represent standard deviations based on averages obtained during the highest power output in three or more separate batch experiments).

tricity generation from the hydrolysate (1,000 mg COD/liter) was produced without a lag phase, and a stable power density of 79.6 mW/m^2 (0.58 V) could be maintained for 12 days. The disappearance of the lag phase for initiation of electricity production, along with the successive transfers of the electrode to new media, suggests that electrons were transferred directly to the electrode from the bacteria attached to the anode. When the electrode was transferred to new medium for the fourth time, a similar power density was immediately obtained, implying that the biofilm formed on the electrode had reached equilibrium. The stable period for power generation with hydrolysate was normally longer than that with other substrates, such as xylose, at the same COD concentration with the same reactor (18). The longer stable period for power generation, which might have been due to the humic acid content of the hydrolysate, showed that hydrolysate was a more suitable fuel for MFCs than xylose.

Power outputs and CEs with different concentrations of hydrolysate. The power generated in the MFC was monitored at hydrolysate concentrations ranging from 250 to 2,000 mg COD/liter (Fig. 2). The maximum power density increased from 65 to 124 mW/m^2 with the initial hydrolysate concentrations from 250 to 2,000 mg COD/liter. Power generation was saturated at hydrolysate concentrations higher than 1,000 mg COD/liter. The open-circuit voltage in the MFC was 0.73 V, and the internal resistance evaluated by the polarization slope method was about 220 Ω. The maximum power output obtained with wheat straw hydrolysate was much higher than those obtained with xylose (38 mW/m^2) and glucose (43 mW/m^2), which were the major constituents of the hydrolysates in two-chamber MFCs (18, 20). The higher power output might be mainly due to the broad organic composition of the hydrolysate, which can easily be utilized by different communities for electricity generation (29, 34). However, this power was lower than the 810 mW/m^2 obtained from corn stover biomass pretreated by steam explosion in a membrane-free single-chamber MFC (46). The lower power density obtained could be due to the higher internal resistance caused by the presence of a membrane and the longer distance between the anode and cathode electrodes in this study (8, 11, 23). The relationship between the hydrolysate concentration and the CE is also shown in Fig. 2. When the initial substrate concentration in-

creased from 250 to 2,000 mg COD/liter, the CE decreased from 37.1 to 15.5%. The differences in CE at different hydrolysate concentrations indicated that some electrons had been consumed by other mechanisms than power generation. With the lower CE achieved in this study, the COD loss was presumably due to biomass generation, incomplete biodegradation of the substrate, hydrogen production, methanogenesis, aerobic degradation, and neutral metabolites diffusing to the cathode chamber (16, 20, 36). Methane and hydrogen were not detected in the headspace in any of the experiments, indicating that methanogenesis and hydrogen production had been effectively inhibited in the system (data not shown). The absence of methane or hydrogen formation indicated that the low CE was not due to the formation of hydrogen and methane.

Substrate degradation and intermediate accumulation. In order to assess the roles of biofilm and suspended bacteria involved in electricity generation, substrate degradation and intermediate accumulation were analyzed in two MFCs operated in different modes. Initially, an MFC was operated in a normal closed-circuit mode. Once there was stable power generation over three batches, the anode chamber was refilled with new medium, while 20 ml of old, suspended solution was transferred to a new MFC with a new anode electrode that was not coated with biofilm to examine substrate degradation and intermediate accumulation (each MFC was fed with 1,000 mg COD/liter hydrolysate). These two MFCs showed the same substrate degradation. The disappearance of xylose and glucose was rapid in both reactors, which resulted in fast formation of acetate (1.8 mM) and other volatile organic acids (less than 0.5 mM) (Fig. 3). However, the time courses of intermediates were distinctly different, especially regarding acetate (Fig. 3). The concentration of acetate in the MFC with the anode electrode coated with biofilm decreased with electricity production (Fig. 3A). In comparison, the concentration of acetate in the MFC with the old, suspended solution was nearly unchanged during the following 10 days of operation (no electricity production) (Fig. 3B). The longer time (more than 12 days) than the normal 3 to 4 days required to produce electricity after the first inoculation might have been due to the different microbial compositions in the inocula used to start up the MFC in this study. Several studies have reported the effect of the inoculum on power production and analyzed the corresponding compositions of electrochemically active biofilms in
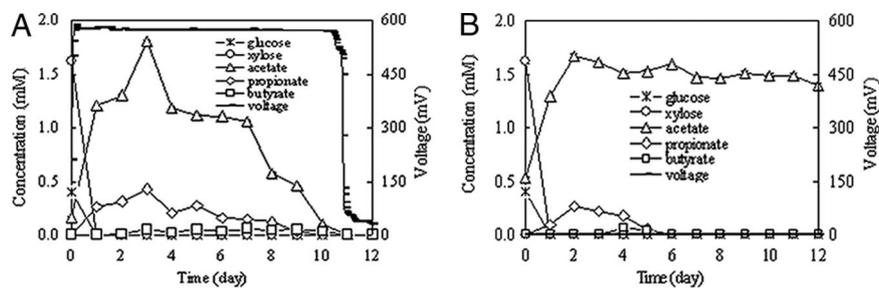
FIG. 3. Substrate degradation and intermediate accumulation as functions of time with (A) or without (B) an anode electrode coated with biofilm.

MFCs (8, 14, 22, 25, 43). There was a far greater diversity of exoelectrogens in these biofilms than was previously suspected, and the community composition to some extent depended on the inoculum (14, 22).

In regard to fermentation, both the suspended bacteria and the biofilm could ferment simple sugars in the hydrolysate to organic acids or alcohols. However, compared to the suspended bacteria, the biofilm might have contained more diverse communities consisting of both electrochemically active bacteria and fermentative bacteria and, consequently, not only fermented simple sugars, but also utilized these fermentation by-products for power generation. While the fermentation process has obviously been observed, the direct utilization of hydrolysate by exoelectrogenic bacteria in general has not been well examined in MFC studies. A previous study showed that *Rhodoferax ferrireducens* can directly oxidize glucose, fructose, sucrose, and xylose to CO_2 with Fe(III) serving as the sole electron acceptor (9). Thus, the possibility that some simple sugars in the hydrolysate might be also utilized directly and completely by some bacteria in the biofilm for electricity production without fermentation cannot be excluded.

Morphological features of biofilm organisms in the hydrolysate-fed MFC. In order to examine the morphology of the anode biofilm, biofilm samples were taken after 36 days of operation with electricity generation and subjected to SEM analysis (Fig. 4). Bacteria of different sizes and shapes were scattered around the electrode, associated with a biofilm formed on its surface. A diverse bacterial community was enriched during this process, and microscopic observation showed an increase in the microbial population and a microbial biofilm attached to the electrode surface with loosely associated microbial clumps (Fig. 4B). Previous studies have proposed that these microbial clumps consist of bacteria that can ferment fuel, such as glucose, into simple fermentation products (19, 22, 26).

DGGE analysis of the microbial community in the MFC. Changes in microbial communities during MFC operation were analyzed by DGGE. The DGGE profiles of biofilm and suspended microorganisms sampled from the MFC at the end of each batch (as shown in Fig. 1) are summarized in Fig. 5. Based on the migration distance, intensities, and similarities between the lanes on the DGGE gel, the banding patterns of the biofilm showed great differences in the first three batch running periods (lanes B1 to B3). The similarities between the lanes were less than 50%. The pattern became stable after the fourth batch and remained unchanged during the following 2 months (lanes B4 to B6). The similarities between the lanes after the third running time were more than 85%. During this
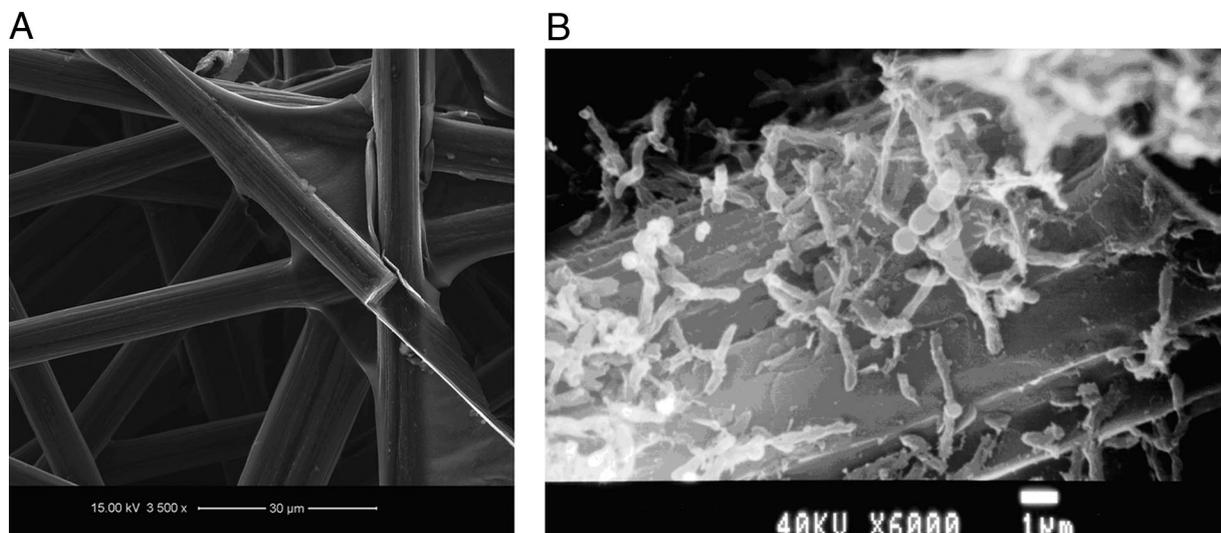

FIG. 4. Morphological features of biofilm attached to an electrode. (A) Carbon paper before use. (B) Electrode after electricity generation from hydrolysate.

changed with time, and electrochemically active bacteria might have been enriched, as indicated by the increase in the power density observed during the successive tests, as shown in Fig. 1. The major bands in the biofilm were different from those in the suspension, indicating that different microbial communities developed in the biofilm and the suspended consortia in MFCs powered by hydrolysates. Most of the bacteria in the original inoculum were absent in the biofilm and suspension, which indicates that a specialized inoculum is needed for successful electrogenesis in MFCs. This is the first report confirming the enrichment of electrochemically active communities using hydrolysate-powered MFCs.

Phylogenetic diversity revealed by cloning and sequencing. In order to provide greater insight into the microbial ecology and diversity, bacterial 16S rRNA gene libraries were examined separately for the biofilm and suspended communities (Table 2). Based on the 16S rRNA gene library analysis, we found that the microbial community in the biofilm of the hydrolysate-enriched two-chamber MFC was dominated by *Bacteroidetes* (40% of sequences), followed by 20% *Alphaproteobacteria*, 20% *Bacillus*, 10% *Deltaproteobacteria*, and 10% *Gammaproteobacteria*. Kim et al. (22) also observed that *Bacteroidetes* and *Gammaproteobacteria* phylotypes were present at higher numbers within libraries from the bacterial clumps and electrode biofilm than in other parts of the fuel cell and suggested that *Gammaproteobacteria* might be involved in current generation. In another study, *Alphaproteobacteria* dominated a community with a river sediment inoculum enriched with a low concentration of glucose and glutamate (39). *Deltaproteobacteria* have been identified as the major bacterial clones in MFCs enriched with acetate (26), and they were believed to be responsible for direct electron transfer to the electrode. In this study, the sequences related to *Deltaproteobacteria* (10% of the sequences) were detected in the biofilm, which also confirmed the involvement of *Deltaproteobac-*
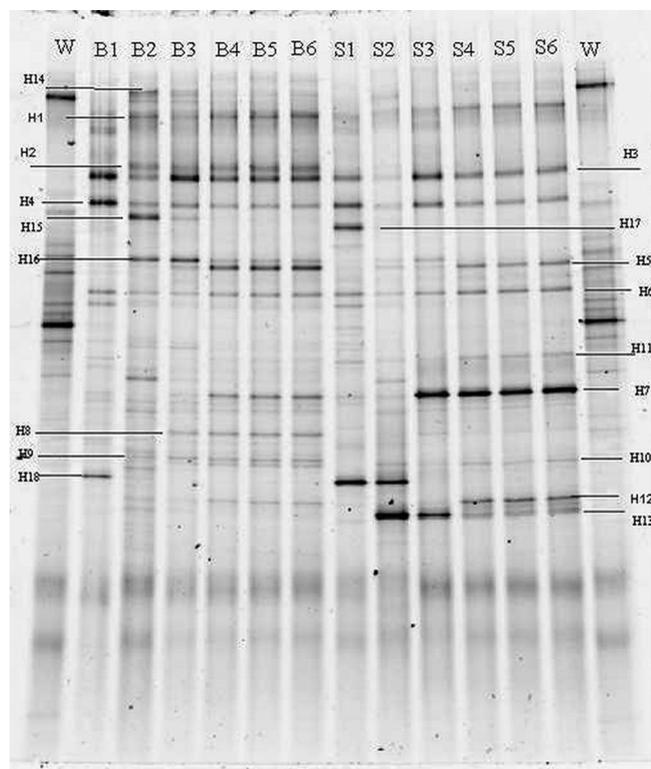

FIG. 5. Anode bacterial community profiles revealed by DGGE. W, wastewater; B, biofilm; S, suspension; H, representative bands.

period, the intensities of some bands became stronger (e.g., bands H1, H5, H7, H8, and H12), while some bands became weaker and even disappeared (e.g., bands H4, H13, H14, H15, and H16). The same trend was also found in the suspended bacterial community. It is clear that the bacterial populations

TABLE 2. DGGE 16S rRNA gene band identifications

Band	Accession no.	Class or genus[a]	GenBank closest match	Identity(%)[b]	Isolation source
H1	FJ222393	*Bacteroidetes*	*D. wimpennyi* ANFA2; AY643492	99	An electrochemically active Fe(III)-reducing bacterium isolated from a microbial fuel cell without a mediator
H2	FJ222394	*Gammaproteobacteria*	*Acinetobacter* sp. strain PD4; EF412969	99	Phenol-degrading bacteria
H3	FJ222395	*Bacteroidetes*	Uncultured bacterium clone 45#2; AY491548	100	Microbial fuel cell enriched with acetate; electrochemically active bacterium
H4	FJ222396	*Alphaproteobacteria*	Uncultured alphaproteobacterium clone BIsi40; AJ318175	100	Waste gas biofilter
H5	FJ222397	*Bacteroidetes*	Uncultured *Bacteroidetes* bacterium clone MB6A-bac2; DQ205192	91	Enrichment culture from an acidic peat bog
H6	FJ222398	*Bacteroidetes*	*D. capnocytophagoides*; U41355	98	Cutaneous abscess
H7/H11	FJ222399	*Bacillus*	Uncultured actinobacterium clone AS42; EU283368	98	Activated sludge from membrane bioreactor
H8	FJ222400	*Deltaproteobacteria*	*G. metallireducens* GS-15; CP000148	98	Complete sequence of *G. metallireducens* GS-15
H9/H10	FJ222401	*Bacillus*	Uncultured bacterium clone PL; DQ191232	97/98	Up-flow anaerobic sludge bed reactor treating peach lye canning effluent
H12	FJ222402	*Alphaproteobacteria*	*Bradyrhizobium* sp. strain KO3D; AB367687	99	Root nodule of the legume *Pueraria montana*
H13	FJ222403	*Betaproteobacteria*	*Azospira oryzae* strain N1; DQ863512	98	Bioreactor

[a] The phylotypes were assigned to phyla based on Ribosomal Database Project II taxonomy classifications.
[b] The values represent the similarities between the associated DGGE band sequence and the closest-match sequence from GenBank.

teria in electricity generation. However, the proportion of *Deltaproteobacteria* in the current study was much lower than that in the literature (70%) for an acetate enrichment study (3). The different medium composition might result in a different bacterial community. Another possibility was that our system might have had too high a redox potential for anaerobic *Geobacteraceae*, due to the oxygen diffusion mentioned above. *Shewanella* species, previously reported to transfer electrons to the surfaces of electrodes via electron-transferring proteins, were not found in either the biofilm or the suspended bacterial samples (2, 5, 41).

BLAST analysis of the gene sequence from band H1 showed 99% identity to *Dysgonomonas wimpennyi* ANFA2, which is an electrochemically active Fe(III)-reducing bacterium isolated from an MFC without a mediator (unpublished data). There was no conventional taxonomic description, precluding any further discussion of its physiological significance. The gene sequence from band H3 showed 100% similarity to uncultured bacterium 45 no. 2, which is an electrochemically active bacterium enriched with acetate in an MFC reported by Lee et al. (26). The gene sequence from band H2 showed 99% sequence similarity to *Acinetobacter* sp. strain PD4, some of which can degrade phenol. *Acinetobacter* spp. can also be enriched in formate-powered MFCs (38). The gene sequence from band H5 showed 98% similarity to *Dysgonomonas capnocytophagoides*, which was also enriched with acetate in a previous study (26). These results indicated that some bacterial species can be enriched in both acetate- and hydrolysate-powered MFCs. Previous studies suggested that members of the *Geobacteraceae*, *Desulfuromonadaceae*, and *Desulfobulbaceae*, related to Fe(III)-reducing bacteria and sulfate-reducing bacteria, could use MFC electrodes as terminal electron acceptors for anaerobic respiration. Several members of these bacterial families, such as *G. sulfurreducens*, *Geobacter metallireducens*, and *Geopsychrobacter electrodiphilus*, have been successfully used in pure culture to generate electricity, which clearly showed that Fe(III)-reducing bacteria could be enriched in MFCs (4, 14, 22). In this study, *G. metallireducens* GS-15 was detected only in the sequence of band H8 from biofilm, indicating that bacteria from band H8 might be involved in electricity generation.

The suspended community, dominated by *Bacteroidetes* (44.4% of sequences), *Alphaproteobacteria* (22.2%), *Bacillus* (22.2%), and *Betaproteobacteria* (11.2%), showed a composition different from that of the biofilm. *Deltaproteobacteria* and *Gammaproteobacteria*, believed to be responsible for the direct electron transfer to an electrode, were not found in the suspended community. *Betaproteobacteria* have been reported as the major class in microbial biofilms in rivers (6). Since band H7 was shown to be a major band in the DGGE patterns of all the suspension samples, the predominant species recovered from the suspended bacteria in the anode chamber appeared to be phylogenetically related (97% similarity) to the genus *Bacillus* of the *Firmicutes*. Previous studies have also reported the presence of *Firmicutes* in the anode-colonizing community of MFCs fed with artificial wastewater containing glucose and glutamate (27%) (37), in acetate-enriched MFCs inoculated with marine sediments (>20%) (4), and in activated sludge (>6%) (26). It was believed that *Firmicutes* might have played the role of converting fermentable substrates (e.g., glucose)

into simple molecules and scavenging oxygen, due to their aerotolerant nature, for power producers and methanogens, a potential example of commensalism (13). This hypothesis was supported by the observation that suspended bacteria from the anode of the MFC produced more reduced metabolites and could not further utilize these metabolites when placed in a new MFC without an anode biofilm.

Comparing the 16S rRNA gene libraries in relation to energy generation, *Bacteroidetes* predominated in the biofilm during operation, indicating that the diversity of iron-reducing and potentially relevant microbes for electricity production might extend beyond the commonly studied *Shewanella* and *Geobacter* species. In general, the microbial diversity found in this study was greater than that found in other MFC studies (20, 26, 32), which were dominated by *Shewanella* and *Geobacter* species. This distinction was probably attributable to the complex composition of the hydrolysate and the wide range of nonselective intermediates and metabolites derived from hydrolysate degradation.

Conclusions. The present study demonstrated that stable power could be generated from wheat straw hydrolysate, which is the most abundant component of plant biomass readily available as a waste material in many parts of the world. The power density reached 123 mW/m^2 with an initial hydrolysate concentration of 1,000 mg COD/liter, while CEs ranged from 37.1 to 15.5%, corresponding to the initial hydrolysate concentrations of 250 to 2,000 mg COD/liter. This is the first study showing that biofilm and suspended microbial communities play different roles in electricity generation from wheat straw hydrolysate. The results imply a potential substrate utilization process in which suspended bacteria and biofilm ferment the complex fuel into simple fermentation products, which can subsequently be utilized by anode electrochemical bacteria to generate electricity. Although suspended cultures have been used for MFC inoculation (28), the suspended culture could not produce electricity from hydrolysate in this study. Mainly fermentation was taking place in this suspended culture. The lack of exoelectrophilic bacteria in the suspended culture was probably due to unfavorable conditions for the growth and proliferation of these cultures. The growth of exoelectrophilic bacteria is probably promoted in an immobilized state on the electrode matrix. Furthermore, a lower stirring rate in the anode chamber probably prevented the immobilized exoelectrophilic cultures from detaching from the biofilm. However, in another study, inoculum scraped from the anode electrode biofilm was found to be effective in electricity generation, underlining the importance of bacterial immobilization on the anode electrode for power generation (24). Further examination will be needed to better clarify the factors affecting the detachment of biofilm in a hydrolysate culture. The microbial community in the anode biofilm was dominated by *Bacteroidetes*, while the suspended bacteria were dominated by *Firmicutes*. Understanding how the microbial community develops and changes over time with wheat straw hydrolysate will assist in the optimization of MFC technology. Further study of the biological factors driving these systems and improvement of MFC design and configuration are required to achieve a technology with practical applications in agricultural and industrial hydrolysate waste utilization.

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