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Bioaugmentation for Electricity Generation from Corn Stover Biomass Using Microbial Fuel Cells

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Corn stover is usually treated by an energy-intensive or expensive process to extract sugars for bioenergy production. However, it is possible to directly generate electricity from corn stover in microbial fuel cells (MFCs) through the addition of microbial consortia specifically acclimated for biomass breakdown. A mixed culture that was developed to have a high saccharification rate with corn stover was added to single-chamber, air-cathode MFCs acclimated for power production using glucose. The MFC produced a maximum power of 331 mW/m^2 with the bioaugmented mixed culture and corn stover, compared to 510 mW/m^2 using glucose. Denaturing gradient gel electrophoresis (DGGE) showed the communities continued to evolve on both the anode and corn stover biomass over 60 days, with several bacteria identified including *Rhodopseudomonas palustris*. The use of residual solids from the steam exploded corn stover produced 8% more power (406 mW/m^2) than the raw corn stover. These results show that it is possible to directly generate electricity from waste corn stover in MFCs through bioaugmentation using naturally occurring bacteria.

Introduction

There is increasing interest in extracting energy from biomass. Most attention has been focused on the possibilities of using cellulosic biomass, such as corn stover, for ethanol production (*1*−*3*). One of the main technical obstacles is that cellulose needs to first be converted to sugars for microbial fermentation to ethanol. This conversion of cellulose to sugars can be accomplished using enzymes or by a steam explosion process (*2*). However, it is possible to directly break down the cellulose using microorganisms to produce hydrogen gas through cellulose fermentation or electricity in microbial fuel cells (MFCs) (*3, 4*).

On the anode of an MFC, bacteria break down organic matter and release electrons to the electrode. Most MFC tests have been conducted using single substrates, but a few studies have examined mixtures of soluble and particulate substrates including domestic and animal wastewaters and corn stover hydrolysates. For example, high power densities (810 to 970 mW/m^2) were achieved by Zuo et al. (*5*) using steam exploded corn stover hydrolysates, where power production was shown to be associated with sugars in the solution. By adding cellulase to the MFC, it is also possible to rapidly convert the cellulose to sugars and produce power in an MFC (100 ± 7 mW/m^2 in a two-chamber MFC) (*6*). Power is also produced from the cellulase added into the MFC. Using the same type of air-cathode MFC used by Zuo et al., 501 ± 20 mW/m^2 was generated from a paper recycling wastewater containing cellulose, as shown by a decrease in the cellulose concentration from 1.18 to 0.051 g/L (*7*). Using rumen microorganisms, 55 mW/m^2 was produced from cellulose (*8*), and a coculture of a *Clostridium cellulolyticum* and *Geobacter sulfurreducens* was used to produce 143 mW/m^2 with carboxymethyl cellulose (CMC) and 59 mW/m^2 with MN301 cellulose in two-chambered MFCs with a ferricyanide-catholyte (*9*).

The direct production of power has not been previously shown directly from a complex source of biomass such as corn stover, which consists of a mixture of cellulose, hemicellulose, and lignin. Unlike purified cellulose, this material can be difficult for bacteria to decompose due to the regular array of the plant cell wall and the difficulty of bacteria accessing cellulose in the presence of the lignin (*10, 11*). Preliminary tests with various inocula such as wastewater microorganisms did not result in an efficient breakdown of corn stover and produced only low current densities in an MFC (Supporting Information, Figure S1). Therefore, we looked for an inoculum that could be used to effectively degrade the corn stover. A microbial community named "H−C" was successfully developed by others at the Harbin Institute of Technology from soil that had a high saccharification rate with cellulose (see the Supporting Information) (*12*). During the breakdown of corn stover with the H−C culture, volatile organic acids and sugars (C_2 to C_6) accumulate in solution. Since these breakdown products would be suitable fuel sources, we examined the use of the H−C culture for electricity generation in an MFC.

Materials and Methods

Corn Stover Preparation and Inocula. Dry corn stover was harvested from a farm in Anda (Heilongjiang, China) and stored for one year. Corn stover was converted into a powder (CSP) using a crushing machine and dried at 105 °C for 2 h. In some tests, steam exploded corn stover (neutral pH steam process; COFCO Co. Ltd. of China) was prepared for comparison to the raw stover. In the steam explosion process 1 kg of dry corn stover produces 10 L of liquid effluent (corn stover liquid; CSL) containing ∼30,000 mg/L of COD, leaving ∼0.8 kg (dry weight) of corn stover residual solids (CSRS) as residual biomass. While the CSL fraction is known to produce power in an MFC (*5*), the CSP and CSRS have not been previously used, and therefore these two materials were examined here as fuels for MFCs. The energy input by the steam explosion process was ∼3418 kJ per kg corn stover added (data from COFCO Co. Ltd., China, for this process). In order to remove any residual sugars or organic acids from the solids produced in the steam explosion process, the CSRS were washed with distilled water three times before being used in tests.

An exoelectrogenic bacterial community was developed on the anode of the MFC using a wastewater inoculum (∼550 mg/L COD) collected from the Taiping municipal wastewater treatment plant (Harbin) and a glucose medium. Frozen

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stocks of H−C culture were revived using filter paper as a substrate, in a modified Hungate medium (see the Supporting Information), with five successive transfers at 30 °C before being used in MFC tests.

MFC Construction and Operation. Seven bottle-type air-cathode MFCs were constructed as previously described (duplicate or triplicate reactors) (*13*) (Supporting Information, Figure S2). Anodes were made of plain carbon paper (2.5 × 4.5 cm; E-TEK, USA), and cathodes were carbon cloth (projected area = 4.9 cm^2; E-TEK, USA) containing Pt catalyst (0.35 mg/cm^2) on the water side and four PTFE layers on the air side (*14*). The total liquid volume of the bottle reactor was 300 mL (250-mL marked bottles; Corning Inc., US). Electrodes were connected to the circuit using titanium wires, and epoxy was used to seal metals exposed to the solution.

The electrolyte was a 50 mM nutrient buffer solution (NBS) with trace minerals and vitamins prepared as previously reported (*13, 14*). Five MFCs were inoculated with domestic wastewater (20%, v/v) and initially fed only glucose (1 g/L) as the substrate (fixed external resistance of 1000 Ω) in a 30 °C constant temperature room. The glucose solution was replaced when the voltage decreased to <50 mV, forming a complete fed-batch cycle that is defined as the cycle time. After similar maximum voltages were observed for two or more consecutive cycles (more than 30 days), all of the MFCs were inoculated with the H−C culture and switched to a different feed. Three MFCs (MFC_{CSP}) were fed with raw corn stover (CSP) (one of these was used only for community analysis) and two MFCs (MFC_{CSRS}) were fed CSRS in 50 mM NBS. After each cycle, 2 mL of the solid residue was removed with a pipet (using a tip cut at the end to increase the diameter of the opening). The remaining solution was discarded, and the 2 mL suspension was added into fresh medium and substrate so that suspended bacteria and those attached to the solid material could be used as an inoculum for the next cycle. Two MFCs were inoculated with only the H−C culture (i.e., no wastewater inoculum) and CSP in order to examine the ability of the H−C culture to produce electricity ($MFC_{CSP,HC}$).

Community Analysis. Bacteria were taken from the anode by removing a piece of the electrode with sterile scissors, from the corn stover residuals in the bottom of the reactor (using a pipet tip with a cut end to increase the tip opening), and the original H−C inoculum, and stored in 50 mM NBS with 25% (v/v) glycerin at −80 °C. Before DNA extraction, the samples were thawed to room temperature and vortexed in sterile NBS to separate bacteria from the electrode or corn stover particles. Cells in suspension were then concentrated by centrifugation (12,000 × g). Total DNA extraction was conducted using a PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the instructions of manufacturer. Amplification of 16S rRNA gene fragments were performed using the primer pairs of 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and GC534R (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGATTACCGCGGCTGCTGG-3′). Denaturing gradient gel electrophoresis (DGGE) was performed using a Bio-Rad Dcode Universal Mutation Detection System (Bio- Rad, Hercules, CA, USA). The denaturing gradient range was 30% to 60% (100% corresponded to 7 M urea and 40% deionized formamide). Samples of PCR products were loaded on gels, which were then run for 6 h at 60 °C (^{150}V). The polyacrylamide gels were stained with silver before analysis using a flatbed scanner (UMAX PowerLook 1000, China). Bands of interest were then cut out from the gels with a sterile knife, mixed with 40 μL of DI water, and incubated overnight at 4 °C. This solution (2 μL) was used amplified with primers 8F and 534R (5′-ATTACCGCGGCTGCTGG-3′). The PCR products were purified with Agarose Gel DNA Purification Kit (Ver.2.0, TaKaRa, Japan) and were ligated and cloned using a DNA Ligation & Cloning Kit (TaKaRa, Japan). Plasmids were isolated from randomly selected clone colonies with MiniBEST Plasmid Purification Kit and sent for sequencing (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., China). The homology searches of sequencing results were performed by using the BLAST server of the National Center for Biotechnology Information along with the BLAST algorithm. The similarity between the DGGE profiles was calculated using the UPGMA (unweighted pairgroup method with arithmetic averages) algorithm in the analysis software for the construction of dendrograms.

Chemical and Electrochemical Analysis. Since the content of protein in corn stover is low (<3%) (*15*), we considered the weight of corn stover sample (W_1, g) as the total weight of cellulose, hemicellulose, and lignin. The total weight of cellulose and lignin in the same sample, W_2 (g), was obtained by acid detergent fiber (ADF) analysis, while the weight of lignin (W_3, g) was measured by the Klasson method (*16*). The cellulose content was calculated as C_c (%) = $[(W_2 - W_3)/W_1] \times 100\%$, the content of hemicellulose C_h (%) = $[(W_1 - W_2)/W_1] \times 100\%$, and the content of lignin C_l (%) = $(W_3/W_1) \times 100\%$. Polarization curves were obtained varying external resistances from 50,000 to 50 Ω, with each resistor tested for 30 min to ensure a stable voltage.

Calculations. Voltages produced by MFCs were recorded every 30 min using a data acquisition system (PISO-813, ICP DAS Co., Ltd.). Current density (i, A/m^2) was calculated according to Ohm's Law $i = V/RA$, where V (V) is the voltage measured, R (Ω) is the external resistance, and A (m^2) is the projected surface area of the cathode. Power density was calculated as P (mW/m^2) = $1000iV$, where 1000 is needed for the given units. The total charge transferred over a complete cycle, Q (C), was calculated as $Q = \int_0^T Idt$, where T (s) is the cycle time, and I (A) is the circuit current at time t (s). The percentages of cellulose (D_c), hemicellulose (D_h), and lignin (D_l) degraded were calculated as $D_c = (W_0C_{c0} - W_fC_{cf})/W_0C_{c0} \times 100\%$, $D_h = (W_0C_{h0} - W_fC_{hf})/W_0C_{h0} \times 100\%$, and $D_l = (W_0C_{l0} - W_fC_{lf})/W_0C_{l0} \times 100\%$, where C_{c0}, C_{h0}, and C_{l0} are the contents of cellulose, hemicellulose, and lignin before degradation, C_{cf}, C_{hf}, and C_{lf} are the contents of cellulose, hemicellulose, and lignin after degradation, and W_0 and W_f are the total solid weight (g) before and after degradation. Energy generated in a complete cycle, E (J), was calculated according to $E = \int_0^T I^2Rdt$. For raw corn stover (CSP), the energy recovery, η (%), was obtained using $\eta = E/(\Delta Hm)$, where ΔH (J/g) is the heat of combustion for corn stover (10,000 J/g), and m (g) is the weight of corn stover consumed in a cycle. For corn stover treatment by steam explosion, the energy needed was calculated as $\eta = (E_1 + E_2)/(\Delta Hm + E_{ex})$, where E_{ex} (J) is the energy consumed during pretreatment (m g raw corn stover), E_1 (J) is the electrical energy generated by CSL (from m g raw corn stover), and E_2 (J) is the electrical energy generated by CSRS (from m g raw corn stover).

Results

Electricity Generation from Glucose During Reactor Startup. Power production was rapidly achieved (~8 d) using glucose as a substrate and a wastewater inoculum. Repeatable cycles of power production were obtained after 30 d, with a maximum power density of 510 mW/m^2 with glucose. When glucose was replaced with CSP (no H−C inoculum), there was little voltage produced (35 ± 5 mV; 2 ± 1 mW/m^2), demonstrating that CSP was not effectively used for power production with this inoculum.

Electricity Generation Using Raw Corn Stover. When the H−C culture was added to the reactors (20%, v/v) in 50 mM NBS, a maximum voltage (330 mV) was obtained from the residual fermentation products transferred with the H−C inoculum within 2 h. After these substrates were consumed, as shown by a drop in the voltage after 3 days, CSP was

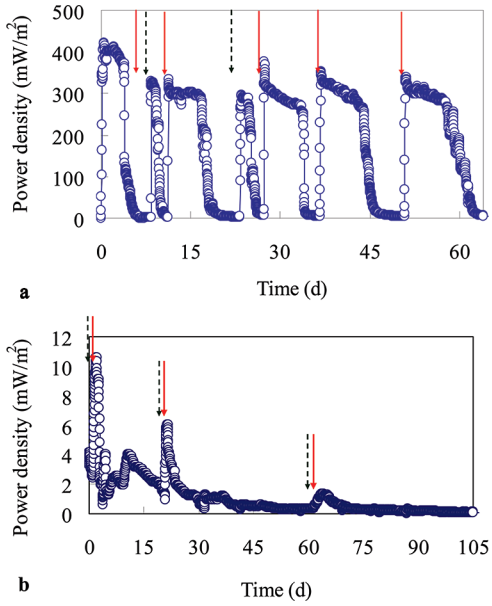

FIGURE 1. Power densities of MFCs with preacclimation (domestic wastewater and glucose) (a) (MFC_{CSP}) and without preacclimation ($MFC_{CSP,\ HC}$) (b). A dotted arrow indicates inoculation with H−C, while a straight arrow indicates the addition of 1 g of raw corn stover.

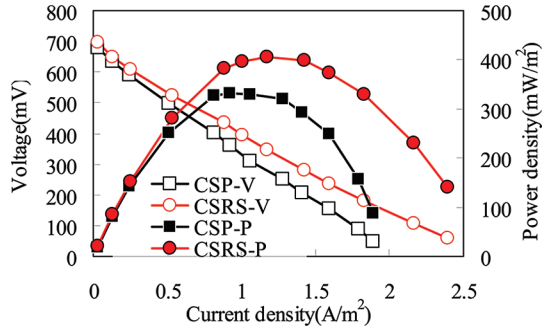

FIGURE 2. Power output and power density curve of MFCs using raw corn stover powder (CSP; MFC_{CSP}) and corn stover residual solids after steam explosion (CSRS; MFC_{CSRS}) as substrate.

added to the (MFC_{CSP}). There was rapid voltage generation that reached a maximum of 384 ± 3 mV (300 ± 5 mW/m^2) in the first cycle (Figure 1a). After the same cycle of voltage output was obtained in the next cycle, the H−C inoculation was discontinued. The next cycles with only CSP in the buffer solution reached maximum voltages of 380 ± 23 mV (296 ± 35 mW/m^2), with a cycle lasting ~14 days. Inoculation of a reactor using only the H−C culture ($MFC_{CSP,HC}$) did not produce substantial power (<10 mW/m^2; peak voltage of 86 mV; Figure 1b).

A maximum power density of 331 mW/m^2 (R_{ex} = 800 Ω) was obtained using CSP by varying the external resistance

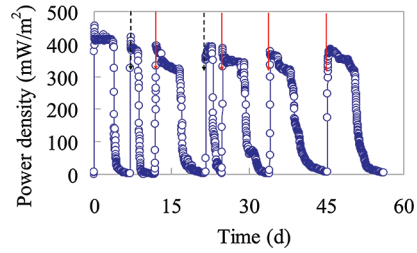

FIGURE 3. Power densities of MFC_{CSRS} using corn stover residual solids after steam explosion.

(Figure 2). Over three cycles of operation, there was an average removal of 42 ± 8% of the cellulose and 17 ± 7% of the hemicellulose (Table 1). There was also evidence that a small amount of lignin was degraded (4 ± 1%).

Electricity Generation Using CSRS. Additional studies were conducted using the solids remaining after the steam explosion process and the H−C inoculum. CSRS contain a much higher proportion of lignin and cellulose than the CSP due to the conversion of the hemicellulose (~50%) into soluble sugars that are removed in the CSL (Table 1) (*17*−*23*). Reactors fed CSRS (MFC_{CSRS}) and inoculated with H−C immediately produced a voltage of up to 437 mV (390 mW/m^2) (Figure 3) and subsequently produced a maximum voltage of 410 ± 20 mV (343 ± 46 mW/m^2) over successive cycles. This maximum power density was 17% higher than that obtained using CSP. In addition, the cycle time was 21% shorter than that obtained with CSP (MFC_{CSP}). Based on polarization data (Figure 2), the maximum power density with CSRS was 406 mW/m^2 (600 Ω, 1.2 A/m^2), which is 23% higher than that obtained using CSP (331 mW/m^2). Overall, cellulose removal with the CSRS was 60 ± 4%, and lignin degradation was 11 ± 4%, with both values larger than that obtained with the CSP (Table 1).

Coulombic and Energy Recovery. Total Coulombic recovery using the MFCs with the CSP substrate was 323 ± 20 C. This was 22% more charge recovered on a mass basis than that achieved using CSRS (264 ± 10 C). An analysis based on total energy recovery showed that 3.6% of the energy in the CSP was recovered. This is more than the 1.6% using the liquid from the steam explosion process (CSL (*29*)) and CSRS based on consideration of the energy used in the steam explosion process (Table 2). Previous results showed that the energy efficiencies with air-cathode MFCs using acetate or butyrate were in the range of 2 to 7.2% based on different external resistance (from 70 to 5000 Ω) (*30*). Thus, energy efficiencies obtained in this study using corn stover were in a similar range as previously obtained using single substrate under conditions of a similar circuit load (1000 Ω).

Community Analysis. DGGE was used to monitor the variation in the microbial communities on both the corn stover biomass in the bottom of the reactor and the biofilm community on the anode (Figure 4, Table 3). The H−C community (H−C Day 0) consisted of bacteria belonging to uncultured *beta proteobacterium* (band 7), *Clostridium* (band 10), and an uncultured bacterium (band 12), consistent with previous analyses of this community (*31*). The initial anode

TABLE 1. Summary of Cellulose, Hemicellulose, and Lignin Contents and Degradation Fractions in Different Corn Stover before and after Electricity Generation

	C_c (%)	C_h (%)	C_l (%)	D_c (%)	D_h (%)	D_l (%)	weight (g)
raw corn stover[a]	59	29	12	-	-	-	1
raw corn stover[b]	49 ± 2	34 ± 1	17 ± 2	42 ± 8	17 ± 7	4 ± 1	0.70 ± 0.07
steam exploded corn stover[a]	68	17	15	-	-	-	1
steam exploded corn stover[b]	48 ± 3	27 ± 3	24 ± 1	60 ± 4	15 ± 4	11 ± 4	0.55 ± 0.03

[a] Before electricity generation. [b] After electricity generation. D_c, D_h, and D_l were degradation (%) of cellulose, hemicellulose, and lignin. The error bars ± SD based on averages measured in three complete cycles.

TABLE 2. Comparison of Total Charge Transferred in a Whole Cycle and Energy Efficiency Using Raw Corn Stover and CSRs Separately as a Substrate in MFCs[a]

		after steam explosion	
	raw corn stover (CSP)	CSRS	CSL (*29*)
m_0 (g) or V_0 (mL)	1.0	0.8	10
Q (C/g)	323 ± 20	211 ± 8	146
E (J/g)	109 ± 7	68 ± 3	59
E_{ex} (J/g)	-	3418	
m (g)	0.30	0.45	
η (%)	3.6	1.6	

[a] m_0 (g) was the weight of solid product after pretreatment, and V_0 (mL) was the volume of liquid product after pretreatment. All these data were calculated under 1000 Ω external resistance. The error bars ± SD were based on averages measured in two complete batches.

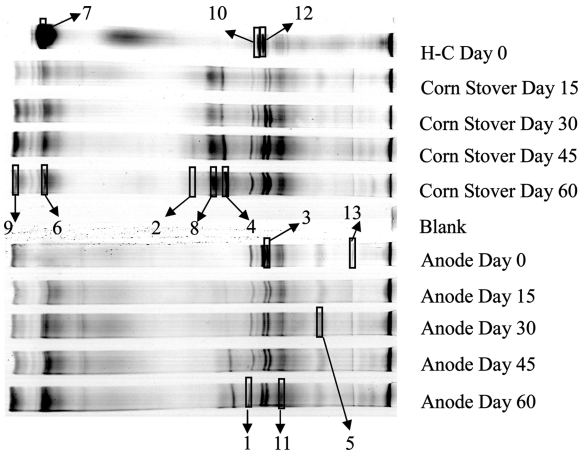

FIGURE 4. DGGE profiles of different bacterial community on the surface of raw corn stover and anode electrode during different time period. 'H−C Day 0' was the original bacterial community of H−C. 'Anode Day 0' was the original bacterial community of anode electrode fed with glucose.

community (Anode Day 0) included *Rhodopseudomonas palustris* (band 1), iron-reducing enrichment clone Cl-A12 (band 3), *Clostridium sticklandii* (band 5), swine manure pit bacterium PPC38 (band 9), uncultured bacteria (bands 11 and 12), and bacterium enrichment culture clone Pav-FEB-Bd24A-C1 (band 13). Fifteen days after H−C was inoculated into the MFC, *Rhodopseudomonas faecalis* (band 6) was found both on corn stover biomass and anode. An uncultured *Verrucomicrobia* bacterium (band 4) and an uncultured bacterium clone ET10-9 (band 8) also were identified on the corn stover bioimass, along with bands 1, 3, 9, and 13 that were previously identified to be associated with the anode community. Further operation after day 15 showed the appearance of an uncultured bacterium clone C23 (band 2) on the corn stover, and bands 4 and 8 (from the corn stover) were detected on the anode on days 45 and day 60.

Discussion

The bioaugmentation of an MFC with a culture acclimated for breakdown of corn stover (H−C) resulted in a maximum power density of 331 mW/m^2. In contrast, little power was produced in tests with a wastewater inoculum (2 ± 1 mW/m^2) or by the H−C inoculum alone (<10 mW/m^2) with raw corn stover (CSP). High power densities were therefore only possible by using the H−C inoculum that was developed to achieve cellulose degradation and saccharification (*12*). Thus, it is likely that the microbes in the H−C inoculum degraded the corn stover and produced soluble fermentation products that were used by exoelectrogenic bacteria on the anode to generate current.

The use of an energy intensive treatment such as steam explosion can improve performance of the MFCs with lignocellulosic substrates, but they are not essential for power generation and overall energy recover is reduced by pretreatment. Treatment of lignocellulose by steam explosion, for example, resulted in a solid residual (CSRS) that could produce a maximum power density of 406 mW/m^2, which is 63% as much energy as that produced with the liquid hydrolysate (CSL; 29) and 23% more than that obtained with the raw corn stover (CSP). This shows that substantial energy remained in the solids that could be recovered in an MFC following the steam explosion process. The degradation of cellulose was also higher in the CSRS (60 ± 4%) than in the CSP (42 ± 8%), likely as a result of the steam-explosion process producing a surface structure of the corn stover residue that aided bacterial degradation (*32*). However, the overall energy recovery was smaller with the CSRS (1.6%) than that obtained with the raw stover (3.6% for CSP) primarily as a result of the inclusion of energy needed for the steam explosion treatment in our analysis (Table 2).

Clustering analysis of DGGE profiles showed that the microbial communities on the surface of the corn stover and the anode changed over time and that differences between these two communities disappeared 30 days after H−C inoculation (Supporting Information, Figure S3). The major shift in the community occurred within the first 15 days.

TABLE 3. Sequence Analyses of Bands Excised from DGGE Gels Derived from Bacterial 16S rDNA Extracted from Raw Corn Stover and Anode Electrode, Arranged on the Basis of Closest Identities[a]

bands	highest homology	identity (%)	accession no.	reference
1	*Rhodopseudomonas palustris*	100	EU221586	*19*
2	uncultured bacterium clone C23	100	DQ088209	unpublished
3	iron-reducing enrichment clone Cl-A12	99	DQ677004	*20*
4	uncultured *Verrucomicrobia* bacterium	99	DQ409967	unpublished
5	*Clostridium sticklandii*	99	M26494	*21*
6	*Rhodopseudomonas faecalis*	99	EU410078	*22*
7	**uncultured *beta proteobacterium***	**98**	**AJ318108**	*23*
8	uncultured bacterium clone ET10−9	98	DQ443965	*24*
9	swine manure pit bacterium PPC38	97	AF445300	*25*
10	***Clostridium intestinale* strain RC**	**95**	**AM158323**	*26*
11	uncultured bacterium clone 68	95	AY324124	*27*
12	**uncultured bacterium clone C50**	**92**	**EU426946**	*28*
13	bacterium enrichment culture clone Pav-FEB-Bd24A-C1	91	EU082063	unpublished

[a] The bands associated with the H−C inoculum are shown with boldface rows.

Pearson correlations of H−C on day 0 versus the corn stover on day 15, and anode on day 0 versus anode on day 15 were 90% and 97%, showing that a larger change took place on the corn stover degradation community than the anodic community in the first 15 days. After that period, the variations of both communities were limited (Pearson correlations >98%), which was coincident with stable power production as shown in Figure 1a.

It was demonstrated for the first time that raw corn stover could be used as a substrate for electricity generation in a single-chambered MFC. Raw corn stover is a complex mixture of cellulose, hemicellulose, and lignin, so that it is much more difficult to be biologically degraded than pure cellulose. The power densities with different corn stover (CSP and CSRS) are still not as high as those achieved with glucose. For example, the maximum power density of glucose was 54% higher than that of CSP. In order to increase power densities and gain additional energy from the biomass, we will need to increase both the efficiency of corn stover saccharification and electricity generation. In addition, two stage processes to optimize the degradation of the lignocellulose and electricity generation in separate systems may be needed to improve overall performance. Furthermore, increases in energy recoveries are needed because the energy recovery in the MFC was much lower than that of cellulosic bioethanol production (66%), or electricity generation by a fired boiler (45%), or gasifier (63%) (assuming the heat of combustion for corn stover is 10 MJ/kg (*33*)).

Acknowledgments

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Supporting Information Available

Additional information on breakdown products produced by culture H−C, example calculation of degradation percentage, peak concentrations of fermentive products using H−C from filter paper in Table S1, power output using corn stover in a wastewater inoculated MFC in Figure S1, a picture of air-cathode bottle MFC in Figure S2, the corresponding dendrogram of DGGE profiles (Figure 4) in Figure S3, and Scanning Electron Microscopy (SEM) images of H−C cells on filter paper and the anode communities that developed in the MFC in Figure S4. This material is available free of charge via the Internet at http://pubs.acs.org.

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