

Removal of volatile fatty acids (VFA) by microbial fuel cell with aluminum electrode and microbial community identification with 16S rRNA sequence

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Abstract—Removal of volatile fatty acids in anaerobic digestion of organic wastes can accelerate eventual decomposition of organic wastes to CO₂ and H₂O using a recovery of electric energy by a microbial fuel cell. The fuel cell anode chamber was a 10 cm (I.D.)×20 cm long cylindrical Plexiglass having an ion ceramic cylinder separator (I.D.10 mm, O.D.12 mm, 0.3 μm average pore size). The aluminum foil cathode (12 cm² surface area) was located inside the ceramic cylinder. Between the two cylinders, 1 liter of activated carbon particles was packed as anode electrode having a void fraction of 0.4. This fuel cell was connected to a 5 liter bioreactor (working volume 1.5 liter), and the bioreactor was run in batch mode by re-circulating a synthetic wastewater of 5 g/L glucose. Maximum TVFA (total volatile fatty acids) and SCOD (soluble chemical oxygen demand) removal rate were 3.79 g/L·day, 5.88 g/L·day, respectively. TVFA removal efficiency (92.7%) and SCOD removal efficiency (94.7%) under maximum current density operation were higher than the operation with maximum power density. In acid fermentation, butyric acid concentration was highest because *Clostridium butyricum* was a dominant microbial community in the inoculum. The microbial cells collected from the anode bio-film samples were affiliated with *Bacillus cereus* based on the nucleotide sequences of dominant DGGE (denaturing gradient gel electrophoresis) bands.

Key words: Microbial Fuel Cell, Acidogenesis, Fermentation, Organic Acid, DGGE

INTRODUCTION

Organic polymers of carbohydrates such as starch or cellulose are slowly decomposed into soluble glucose or oligomers and then converted into various fatty acids such as acetic acid, propionic acids, butyric acids, etc. These VFAs are converted anaerobically to carbon dioxide and methane gas, which is quite a rate-limiting step. In order to accelerate the removal rate of organic pollutants from aqueous phase, it is necessary to remove VFAs from the aqueous phase as soon as possible rather than to let them go through methane-forming step. Fig. 1 shows various strategies of shortening anaerobic digestion step and its utilization of VFAs [1-6].

VFAs are not only a good substrate for PHB production [4], electron donor in the denitrification [5], but also can be used in generating conventional biogas that can be further reformed to hydrogen for PEM (Proton Exchange Membrane) fuel cell [6]. However, the biogas formation step is rather more rate limiting than the rapid acid forming step. This requires a relatively long retention time, which requires huge bioreactor volume. Furthermore, direct burning of wet organic wastes is not feasible owing to the generation of deadly toxic dioxin gas. If we can accelerate a slow methane-generating step to some extent, perhaps the only feasible solution would be to let organic wastes go through anaerobic acidification steps.

We have considered the use of chemical or microbial fuel cells in treating organic wastes from the aqueous phase as a way of bypassing biogas formation step. One way is to oxidize VFAs directly

into CO₂ or H₂O by using microbial or chemical fuel cells. Since chemical fuels are not much suitable for removing VFAs, we consider here use of microbial fuel cells.

MFC (microbial fuel cell) is a device that uses bacteria as the catalyst to oxidize organic or inorganic matters and generates current [7-11]. Electricity generation is supported by a range of biodegradable substrates, including glucose, acetate, lactate, butyrate, ethanol and organic matter in wastewater [12,13]. An MFC generally consists of two chambers, one anaerobic (anode) and the other aerobic (cathode). In the anaerobic chamber, substrate is oxidized by bacteria and the electrons transferred to the anode either by an exogenous electron carrier [14], or mediators [15,16], or directly from the bacterial respiratory enzyme [17-19] to the electrode. In the latter case, the MFC is known as a mediator-less MFC. Bacteria in that system typically have electrochemically active redox enzymes such as cytochromes on their outer membranes that can transfer electrons to external materials [17]. The anaerobic chamber is connected internally to the aerobic chamber by a proton-conducting material, and externally by a wire that completes the circuit. In the aerobic chamber, electrons that pass along the circuit combine with protons and oxygen to form water.

Several factors affect performance of MFC including the microbial inoculum, chemical substrate (fuel), type of proton exchange material (and the absence of this material), cell internal and external resistance, solution ionic strength, electrode materials and electrode spacing [20-22]. The cathode is an important factor in the performance of an MFC due to the poor kinetics of the oxygen reduction reaction in a neutral pH medium [17]. Furthermore, the need for oxygen at the cathode is a disadvantage to operation of the sys-

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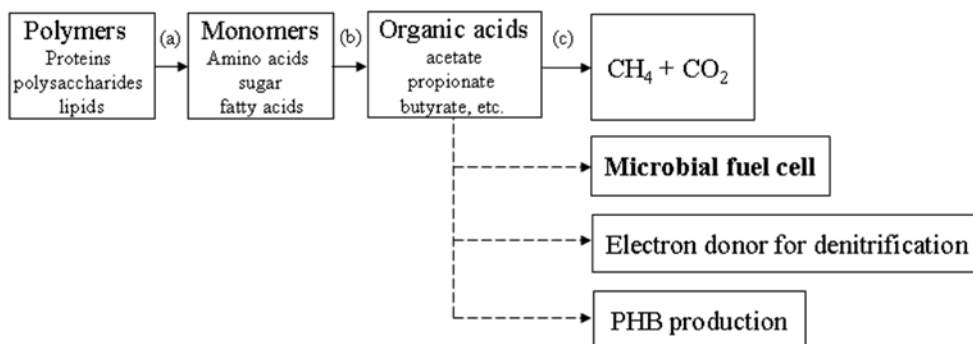


Fig. 1. Various strategies of shortening anaerobic digestion step and its utilization of volatile fatty acids. Solid line indicates typical anaerobic digestion and dotted line indicates alternative strategies without methane formation. (a) Hydrolysis, (b) acidogenesis or acetogenesis, (c) methanogenesis.

tem because oxygen can leak into the anode chamber and either lower energy recovery or inhibit the growth of obligate anaerobes. The cathode reaction can be improved through electrode modification with metals, surfactants, organic materials [23], or by using cathode mediators such as methylene blue, viologens, thionines, ferricyanides, and quinoid compounds [24,25]. Another problem is cathode overpotential in the MFC. In practice, the maximum open circuit potentials (potential observed when no current is running through the MFC electrical circuit) observed is of the order of 750–800 mV [26]. Upon closure of the electrical loop, this voltage decreases significantly, mainly because of the so-called overpotentials, which are potential losses owing to electron transfer resistances and internal resistances. Three kinds of overpotentials can be defined: activation overpotentials, ohmic losses and concentration polarization [27]. In MFCs, the activation overpotential appears to be the major limiting factor. This overpotential is largely dependent on the current density flowing through the anode, the electrochemical properties of the electrode, the presence of mediating compounds and the operational temperature.

In this study, we examined that electrochemical microbial removal of volatile short chain fatty acid with aluminum electrode as the cathode. Aluminum is an abundant element in the Earth's crust and it can be easily reduced in the presence of hydrogen ion because of its reactivity with acid [28]. Also, we identified microbial communities on the anode electrode by DGGE.

MATERIALS AND METHODS

1. Microorganisms and Medium

Activated sludge in an anaerobic digester was used as the inoculum and was treated by sterilization at 90 °C for 15 minutes prior to use. In acid fermentation, the artificial wastewater consisted of (per liter of deionized water): glucose, 5.0 g; NH_4Cl , 0.2 g; $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 0.15 g; KCl, 0.33 g; NaCl, 0.30 g; MgCl_2 , 3.15 g; K_2HPO_4 , 1.26 g; KH_2PO_4 , 0.42 g; trace metals (1 mL) and yeast extract (0.25–1.0 g) [29].

2. MFC Construction, Electrodes and Separator

The MFC was constructed of an up-flow type bioreactor and manufactured with 10 cm (I.D.) \times 20 cm long cylindrical Plexiglas, and the working volume of anode chamber was 1 liter, including a void volume of 400 cm^3 (Fig. 2). A hollow fiber ceramic cylinder (I.D.

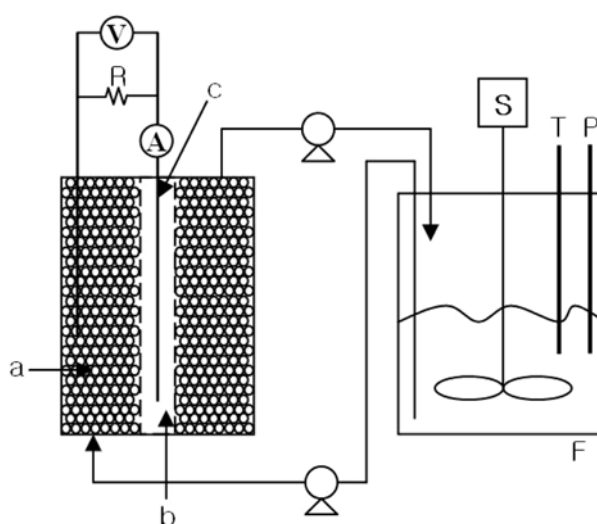


Fig. 2. Schematic diagram of the MFC in this study. a: Activated carbon (anode), b: Hollow fiber ceramic membrane (ion separator), c: Aluminum foil (cathode), T: Temperature sensor, P: pH sensor, S: Agitation controller, F: Fermentor (5 liter), R: Resistor, V: Potential sensing unit, A: Current sensing unit.

10 mm, O.D. 12 mm) with 0.3 μm average pore size was inserted as an ion separator in the MFC. Between the two cylinders, 1 liter of activated carbon particles with 1 mm average granular size was packed as anode electrode. Aluminum foil (12 cm^2 surface area, Lotte Aluminium Co., Ltd., Korea) was used as cathode electrode in the ceramic cylinder. Current collecting was done by connecting the end of each electrode and Platinum wire.

3. MFC Operation

This fuel cell was connected to a 5 liter bioreactor (working volume 1.5 liter), and the bioreactor was run on a batch mode with an artificial wastewater of 5 g/L glucose at 35 °C and pH 6.0. Effluent of the anode chamber was re-circulated to the bioreactor by peristaltic pump, and up-flow linear velocity of the anode chamber was kept at a constant flow rate of 1 m/h.

4. Analytical Methods

The concentration of COD (chemical oxygen demand), SCOD (soluble COD) was determined according to the procedures described

in Standard Methods [30]. The concentration of each VFA (acetic acid, propionic acid, butyric acid) and glucose was measured by high performance liquid chromatography (Hitachi L-3300 RI detector, Tokyo, Japan) equipped with an ion exchange column (Aminex HPX-87H, Hercules, CA, USA) by using 5 mM H₂SO₄ as mobile phase. All samples were filtered through a 0.22 μm (pore diameter) membrane filter prior to measurement.

5. Electrochemical Measurement and Calculation

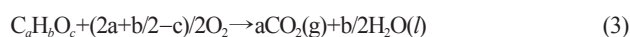
The system was monitored with a precision multimeter and data acquisition system (Model 2700, Keithly Instruments, Inc.). Power (P) was calculated according to P=IV, where I (C/s) is the current, and V (V) is the voltage. The coulombic efficiency, C_{Efficiency} (%), was calculated as C_{Efficiency} (%) = C_p/C_{ti} × 100%, where C_p is the total coulombs calculated by integrating the current over time. C_{ti} is the theoretical amount of coulombs that can be produced from substrates (acetate i=a, propionate i=b, butyrate i=c), calculated as.

$$C_{ti} = Fb_i S_i / M_i \quad (1)$$

where F is Faraday's constant (96,485 C/mol of e⁻), b_i is the number of moles of electrons produced per mole of substrate (b_a=8, b_b=12, b_c=20), S_i is the substrate concentration and M_i is the molecular weight of the substrate. Overall energy recovery was calculated as, E_{Recovery} (%) = E_p/E_{ti} × 100%, where E_p (J) is the total energy calculated by integrating the power over time. E_{ti} (J) is the theoretical amount of energy that can be produced from substrate, calculated as,

$$E_{ti} = \Delta H S_i / M_i \quad (2)$$

where ΔH is the enthalpy change of the following reaction under standard conditions:



This is the oxidation reaction of organic matters to calculate standard enthalpy changes.

6. Microbial Community Analysis

The samples of MFCs were obtained from activated sludge. Total DNA of samples was extracted by using an UltraClean™ Soil DNA Isolation Kit (Mo Bio Labs. Inc., Solana Beach, CA). The extracted DNA was used as template in PCR. For DGGE analysis, 16S rRNA gene fragments were amplified with PCR primers 341f-GC and 518r [31]. PCR mixtures contained 1 μl of template DNA and BIONEER AccuPower® PCR premix (Taq polymerase 1 U, dNTP 250 μM, Tris-HCl [pH 9.0] 10 mM, KCl 40 mM, MgCl₂ 1.5 mM, stabilizer and tracking dye). Agarose gel 1.5% (w/v) electrophoresis was used to confirm PCR products of correct size. DGGE condition was optimized by using a DCode system (Bio-Rad, Hercules, CA). PCR products were loaded on a 10% (w/v) polyacrylamide gel (acrylamide: N,N'-methylenebisacrylamide, 37.5 : 1, Bio-Rad) in total 7 L of buffer compounding 140 mL of 50× TAE and sterile distilled water. DGGE was operated at 60 °C for 4 h [32,33]. The denaturing gradient in the gel was generated by mixing two stock solutions of 10% polyacrylamide containing 40% and 60% denaturant, denaturing gradient increased in the direction of electrophoresis. After electrophoresis, the DGGE gel was stained with GreenStar™ (Bioneer Co., Daejeon, Korea) for 40 min and visualized on a UV transilluminator. Major DNA bands were excised from DGGE gels and re-amplified by PCR for nucleotide sequencing, and nucleotide

sequences were described previously [34].

RESULT AND DISCUSSION

1. Removal of Volatile Fatty Acids

The MFC was operated in a batch mode, connecting the acid fermentor to control constant temperature and pH. Effluent from the bioreactor was reintroduced to the fermentor and circulated at a flow rate of 10 mL/min by the peristaltic pump. To immobilize cells on the activated carbon anode, the external electrical loop between the anode and cathode was connected with minimum external resistance (5-10 Ω) for about 5-10 days. After that, the acid fermentation broth was changed with artificial wastewater, 5 g/L of glucose concentration. The open circuit potential (OCP) reached up to 0.65 V and current was produced after the external resistor was connected. The characteristics of electricity generation were examined by a polarization curve, which showed that the maximum power density of 91 mW/m³ (cathode chamber volume) occurred at 100 Ω (0.38 V). Fig. 3(a) shows removal rate profiles of each VFA component in the MFC. According to the proceeding of the acid fermentation with glucose as a carbon source, acetic acid increased from 0.21 g/L to 1.70 g/L for the initial 18 hours and decreased to 0.80 g/L for the last 24 hours. Removal rate of acetic acid was 0.91 g/L·day. During the fermentation and current generation, lactic, succinic and formic, propionic and butyric acids slightly increased up to about 0.2 g/L (data not shown), but removed completely. TVFA and SCOD removal rate was 1.00 g/L·day, 2.79 g/L·day, respectively. TVFA removal efficiency (55.8%) was less than SCOD removal efficiency (77.1%), because the most removed component was glucose by acid fermentation and current generation.

To evaluate removal characteristics of acids mixture without glucose, three kinds of main product (acetic acid/Ac, propionic acid/Pc, butyric acid/Bc) in acid fermentation were supplied in the reactor. Initial concentration of acids mixture depends on microbial community in acid fermentation; in this case, they are 2.79 g/L of Ac, 0.23 g/L of Pc, 4.65 g/L of Bc, respectively. Butyric acid concentration was the most high, because most cells in acid fermentation were identified as *Clostridium butyricum* by DGGE, which will be discussed later. The OCP reached 0.63 V, which was similar to the first case, and maximum power density was 30.9 mW/m³ (cathode chamber volume) which occurred at 10 Ω (0.07 V) with maximum current density mode. Fig. 3(b) shows removal rate profiles of acid mixture. Butyric acid removed rapidly for initial 10 hours. The removal rate of butyric acid decreased from initial 16.34 g/L·day to 0.32 g/L·day and average removal rate was 2.46 g/L·day. Acetic acid was removed steadily and removal rate was 1.22 g/L·day. Finally, acetic acid still contributed to generation of current after butyric acid was removed completely. TVFA and SCOD removal rate was 3.79 g/L·day, 5.88 g/L·day, respectively. TVFA removal efficiency (92.7%) and SCOD removal efficiency (94.7%) were higher than operation with maximum power density mode; these results show that we should operate MFC under maximum current density mode with minimum external resistance to remove wastewater something like VFAs in acid fermentation.

2. Evaluation of Electrochemical Characteristics

To estimate efficiency of each experiment, the overall energy recovery and the coulombic efficiency were calculated by equations

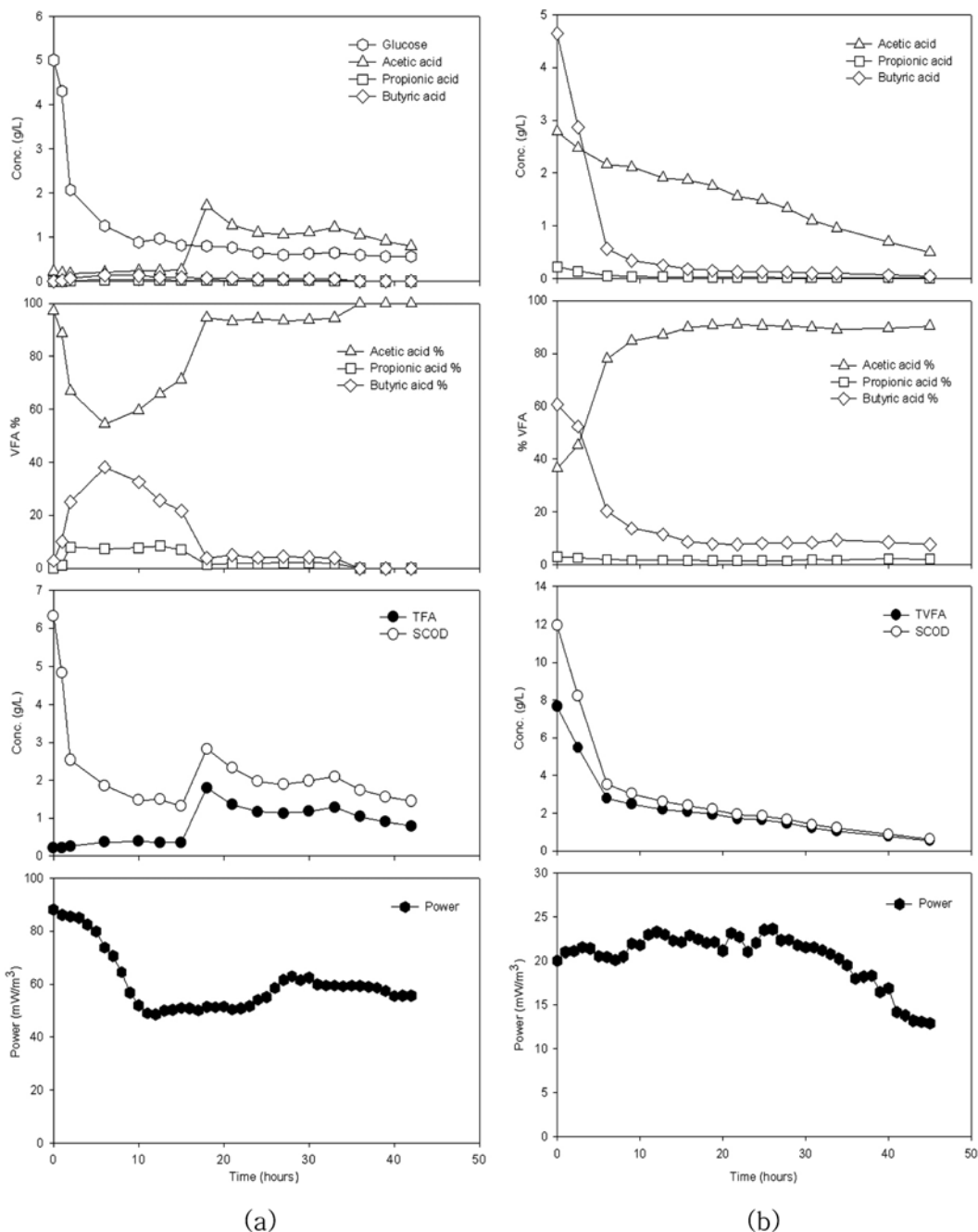


Fig. 3. Removal characteristics of acid mixture in the MFC. (a) is under maximum power density mode, (b) is under maximum current density mode.

as mentioned in the materials and methods section. We assumed that all electrons from aluminum were delivered to diffused hydrogen ions through the separator. Coulombic efficiency decreased from 48.6% to 25.7% when external circuit resistance decreased from 100 Ω to 10 Ω . This resulted from using different kinds of substrate and concentration in each experiment. In general, coulombic efficiency was a function of substrate concentration and circuit resistance, and increasing circuit resistance or substrate concentration results in a decrease of coulombic efficiency, because it is difficult to recover electrons from substrate with higher external resistance. Furthermore, electricity could have been generated from butyric acid

degradation to acetic acid according to $C_4H_8O_2 + 2H_2O \rightarrow 2C_2H_4O_2 + 4H^+ + 4e^-$. Butyric acid could be first converted into acetic acid by butyrate-degrading acetogenic bacteria [35] and contribute to generate current.

While coulombic efficiency indicates recovery of electrons, the overall energy recovery of the system represents the energy harvested as electricity from bacteria versus that lost to other processes. Under the maximum current density operation, 2.2% of the energy was recovered at initial 5 g/L of glucose concentration. Energy recovery was increased from 2.2 to 34.5% by increasing circuit resistance (from 10 to 100 Ω) for lower circuit loads. Increasing the re-

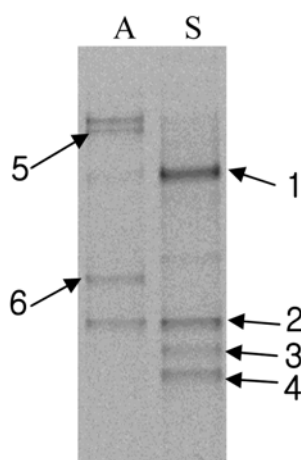


Fig. 4. DGGE profile of 16S rRNA gene fragments. The fragments were PCR-amplified from the total DNA extracted from MFCs. A: anode of MFC, S: activated sludge. Each band was identified in Table 2 and Fig. 4.

Table 1. Summary of removal rate and efficiency

Reaction condition	Fuel	Glucose, Acid mixture	Acid mixture
	Electrical mode	Maximum power density	Maximum current density
Glucose (%/gL ⁻¹ day ⁻¹)		88.7/2.54	-
Acetic acid (%/gL ⁻¹ day ⁻¹)		53.3/0.70	82.0/1.219
Propionic acid (%/gL ⁻¹ day ⁻¹)		100/0.03	95.1/0.114
Butyric acid (%/gL ⁻¹ day ⁻¹)		100/0.11	99.1/2.457
VFAs (%/gL ⁻¹ day ⁻¹)		55.8/1.00	92.7/3.787
SCOD (%/gL ⁻¹ day ⁻¹)		77.1/2.78	94.7/5.876
C _{Efficiency} (%)		48.6	25.7
E _{Recovery} (%)		34.5	2.2

sistance further to 1,500 Ω in VFA-fed cells decreased the overall energy recovery to 0.1% (data not shown), because cell potential decreased to 0.001 V or negative.

In this study, electrochemical removal of VFA was done by using aluminum electrode rather than conventional carbon electrode, but that metal electrode has some critical problems that have to be considered or solved for reaction design. First, it can be oxidized quickly and forms a thin layer on itself when the metal is exposed to air.

This thin layer of oxidation might interrupt electrochemical reaction, because that already changed to oxide form with low reactivity. Second, aluminum can be precipitated to various salt forms such as aluminum acetate. Even though they could be important for material application or aluminum salt production, they are just a by-product in this case. Furthermore, once it changes to salt forms, it is difficult to regenerate it. In acids circumstance, metal can produce hydrogen gas and aluminum salts. For example, aluminum and hydrochloric acid can easily produce aluminum chloride and hydrogen gas. However, we could complete several sets of experiments for seven days without changing aluminum foil.

3. Microbial Community on the Anode Electrode

After MFC operation, immobilized cells on anode electrode were collected to identify microbial communities. The PCR-amplified 16S rRNA gene fragments were used for DGGE. 16S rRNA of sludge, anode bio-film showed at size of about 200 bp band in each column. They were separating the PCR-amplified 16S rRNA through DGGE (Fig. 4). In the anode bio-film samples, nucleotide sequences of dominant DGGE bands were affiliated with *Bacillus cereus*. *Bacillus* species are either obligate or facultative aerobes, but similar strain had been used in MFC; in that case, a thermophilic strain was used as a biocatalyst [36].

The profile of the microbial culture inoculated into the MFCs was different from that observed with anode bio-film. In the activated sludge sample, nucleotide sequences of dominant DGGE bands were affiliated with *Clostridium butyricum*, *Nitrosospira* sp. and *Pseudomonas* sp., which means that those microbial communities had been washed out from in the MFC during the operation. Fig. 5 illustrates the phylogenetic tree of the four species identified in this study and their respective close relatives based on partial 16S rRNA sequence. By comparing the RNA sequence of these four populations with those available in the GenBank, the predominant band 1 was most like a member of genus *Clostridium*, resembling *Clostridium butyricum* with 100% RNA similarity from with 169 nucleotide comparison. *Clostridium butyricum* is a genus of Gram-positive bacteria and are obligate anaerobes found in the wastewater treatment system, including activated sludge. *Nitrosospira species* is a member of nitrifying bacteria, which are able to grow chemolithotrophically at the expense of inorganic nitrogen compounds. Nitrifying bacteria are widespread in soil and water, and are found in highest numbers where considerable amounts of ammonia are present (areas with extensive protein decomposition, and sewage treatment plants). Also, *pseudomonas species* was found in the activated sludge sample.

4. Biomass as Alternative Carbon Source

Table 2. Characteristics of 16S rDNA fragments obtained from DGGE gel

DGGE band ^a	Closest match	Sequence similarity (%)	Accession number ^b
1	<i>Clostridium butyricum</i> strain DSM 523	100	AJ458421
2	<i>Clostridium butyricum</i>	99	AY604563
3	<i>Nitrosospira</i> sp.	100	AJ005543
4	<i>Pseudomonas</i> sp. 10-Eb	96	AY770008
5	<i>Bacillus cereus</i> isolate A4s3	100	DQ058169
6	unidentified bacterium	91	AJ842284

^aName of the DGGE bands in Fig. 4

^bNCBI Accession Number in the GenBank

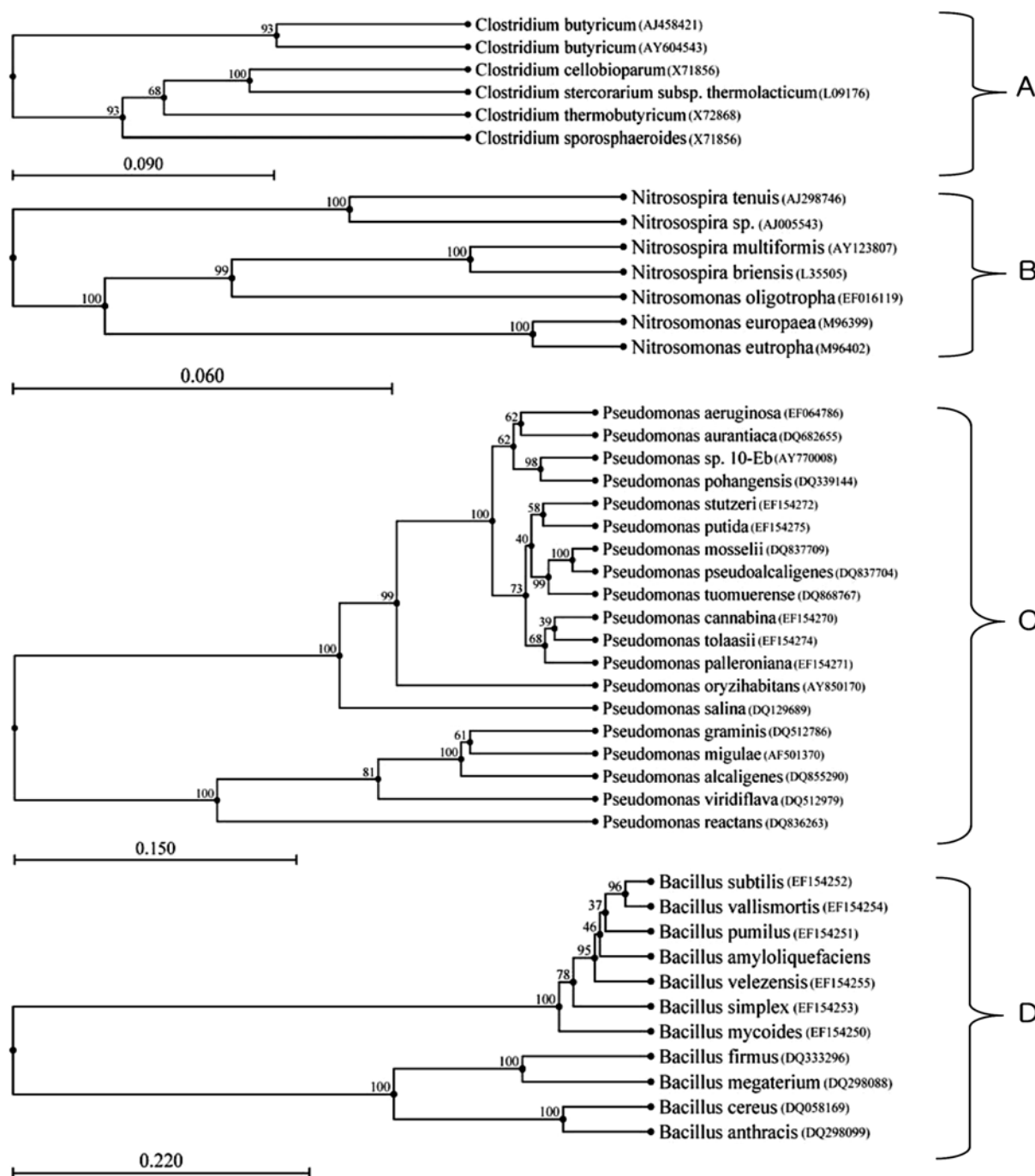


Fig. 5. The phylogenetic tree of the four species identified in this study and their close relatives in the classes *Clostridia* (A), Beta proteobacteria (B), Gamma proteobacteria (C), *Bacilli* (D), based on the 16S rRNA sequence. The tree based on the Jukes-Cantor distance was constructed by using the neighbor-joining algorithm with 500 bootstrappings. The scale bar represents substitution per nucleotide position. Numbers at the nodes are the bootstrap values.

Biomass refers to living or recently dead biological material that can be used as fuel or for industrial chemical production. It may also include biodegradable wastes that do possess much value as is. Oils, natural gas or coal are bound to become depleted eventually, and furthermore they generate global warming carbon dioxide. For this reason, renewable biomass is preferred to fossil-based carbon sources as energy and chemical raw materials. The chemical energy harnessed in biomass is released as heat by generating

carbon dioxide gas, but when biomass crops are grown, a nearly equivalent amount of carbon dioxide is captured through photosynthesis [37].

Chemical fuel cells use hydrogen gas for PEMFC, carbon monoxide, methane for solid oxide fuel cell, methanol for direct methanol fuel cell [38]. These fuel sources, in whatever form these take, are all derived from fossil fuel and generate global warming carbon dioxide gas. However, biomass itself does not have much value as

fuel unless it is converted to liquid soluble glucose, xylose or volatile fatty acids. Once biomass is converted to a monomer form, then microbial cells can metabolize to convert them directly to electricity. However, the energy density generated by microbial cells amounts only to 1/1,000-1/100th of comparable chemical fuel cells and thus it is not very practical as a power source. Perhaps it may qualify as biosensors. One way of converting biomass to energy is to reform its soluble glucose or volatile fatty acids to hydrogen gas. This would be much faster than microbial hydrogen production.

CONCLUSION

Electrochemical removal of volatile acids in the MFC was successfully performed by using corrosive aluminum electrode. Removal rate of TVFA and SCOD with low external resistance increased more than experiment under maximum power density operation. It was shown that the aluminum electrode was corrosive in the cathode chamber or precipitated to salt form, aluminum acetate, but unique characteristics easily give electrons to diffuse hydrogen through a separator, which improved the VFA removal efficiency and decreased cell potential. By comparing 16S rRNA sequence, dominant microbial communities were identified in the MFC and activated sludge.

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