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POTATO WASTE TREATMENT BY MICROBIAL FUEL CELL. EVALUATION BASED ON ELECTRICITY GENERATION, ORGANIC MATTER REMOVAL AND MICROBIAL STRUCTURE

The performance of microbial fuel cell (MFC) in treating potato waste was evaluated using a two-chamber MFC supplied with potato liquid after mastication of market available fresh potato. Evaluation was conducted based on electricity generation, organic matter removal (COD_{Cr}, DOC and volatile fatty acids (VFAs)), and microbial structure on the anode and in the anodic solution of the reactor. Current density exhibited a trend that followed the concentration changes of organic matter in the solution, with its highest value being observed as 208 mA/m². Effective removal of organic matter was also observed. By the end of the experiment, the removal for total COD reached about 84%. Bacterial structure analysis based on PCR, DGGE and sequencing indicated that more species were developed in the anodic solution than on the anode, with Proteobacteria, Firmicutes and Bacteroides being dominant. Geobacter, a well reported exoelectrogenic species, was found more predominant on the anode than in the anodic solution. The results thus indicated that simultaneous stabilization and electricity generation could be achieved when potato waste is treated in MFC.

1. INTRODUCTION

Vegetable wastes containing high content of organic matter are generated in the processes of harvesting, storage, transportation, marketing and processing. Proper treatment of vegetable wastes is indispensable for protection of our living environment and natural environment since, without proper treatment, vegetable wastes can release foul

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smells and leachates during their decaying that contaminate air, water and soils. Some of their decaying products can even threaten our food safety and health. In most developed countries, vegetable wastes are mainly treated, together with other burnable municipal wastes, through incineration. In developing countries, however, either landfill or direct dumping remains as the major method used for dealing with such wastes. For all these methods, the rich content of organic matter contained in the wastes is not used or recycled. The concept of reuse, recycling and resource recovery requires consistent seeking for effective and sustainable approaches beyond these methods and also beyond some environment friendly practices applied still in small scales such as the use as livestock feeds after simple treatment, composting and anaerobic digestion.

Microbial fuel cell (MFC) which has been studied mainly for wastewater treatment, is probably a promising method for realizing the purpose of destabilization of organic wastes. The major merit of this method is its capability to convert chemical energy of organic substrates into electricity through catalyzing the anaerobic oxidation process in the anodic chamber by bacteria as the catalyst [1]. This merit has also been reported by several studies treating such organic wastes as cattle dung, composite food waste and sewage sludge [2–4]. Following this trend, a few studies using vegetable wastes as the substrate of MFC were also conducted. For example, using the extract of the market available composite vegetable waste as the substrate, Venkata Mohan et al. [5] investigated the electricity generation by a single-chamber MFC reactor. In another study that used clover sap as the substrate of MFC, Clauwaert et al. [6] achieved a maximum current density of 193 A/m³ and a maximum power density of 70 W/m³. In a recent study, Zhang et al. [7] found that corn stover could be used to remove sulfide and generate electricity in MFC, with the maximum power density reaching 744 mW/m², the maximum sulfide removal of 91% and the maximum COD removal of 52%. The composition of vegetable waste differs with the dietary habits and regions, and various vegetable types may respond differently in the reactors of MFC. Taking into consideration that potato is a widely planted and distributed vegetable type in the world and that its waste may constitute for a big percentage of the total vegetable wastes from markets, restaurants and households, it is very important to investigate the treatability of potato by MFC before composite vegetable wastes are considered for treatment. Although information on the exact percentage of potato in the total vegetable waste is not available, according to the Statistics Division of the Food and Agriculture Organization of the United Nations, the total potato production in the world reached about 368 million tons in 2013⁵.

In MFC, a complex metabolic mechanism of microbes, notably for bacteria, is involved in electricity generation and organic matter decomposition. Some bacteria such as *Synechocystis* sp. can produce electrically conductive appendages (called also as nanowires), through which electrons are able to be transferred to the anode, as reported by

⁵<http://faostat3.fao.org/browse/Q/QC/E>

Logan [8]. Moreover, some fermentative bacteria attached on the anode such as *Geobacter* sp. [9], *Aeromonas hydrophila* [10] and *Clostridium butyricum* [11], can also transfer electrons in addition to their capability for electron generation during degradation of organic matter either on the anode or in the anodic solution. Regarding microbial community populated in the anodic chamber of MFC, intensive literature review reveals that, most previous studies focused only on its presence on the electrode [2, 9], with studies focusing also on the microbial composition and structure inside the anodic solution are very limited. Bacteria populated in the anodic solution are also important since they may directly involve in breaking down complex organic matter into species easier for use by electricity generating bacteria.

Therefore, the main objective of this study was to investigate the performance of MFC for treatment of potato waste. For this, a two-chamber MFC was used as the reactor and potato liquid obtained by masticating fresh potato from a market was used as the waste to be treated. The performance was evaluated in terms of electricity generation, organic matter removal and microbial structure both on the anode and in the anodic solution of the reactor. For evaluation of the organic matter removal, in addition to chemical oxygen demand (COD_{Cr}) and dissolved organic carbon (DOC), the formation and dissipation of volatile fatty acids (VFAs) during treatment were also measured. Microbial structures were evaluated through analysis of the extracted bacterial 16S rDNA with quantitative polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE) and sequencing. The reason for using a two-chamber MFC as the reactor for this study is because, even if the efficiency of this type of reactor is generally considered lower than single-chamber MFC, in many recent studies it is still used for better investigation of fundamentals of reaction mechanisms and performance, and also for development of more efficient electrode materials [5, 12, 13]. The cathode chamber can also allow simultaneous removal for some substances based on either oxidative reactions via aerobic bacteria or reductive reactions via electrons transferred from the anode chamber, such as ammonium or its oxidized product of nitrate in wastewater [14].

2. MATERIALS AND METHODS

Preparation of potato waste. Fresh potato product obtained from a local supermarket (Kanese Supermarket, Gifu city) was masticated with an electrical blender and then filtered through a membrane filter with the pore size of 3 μm . The resulting potato liquid was used after conducting necessary dilution to make the working solution to be added to the anodic chamber of the MFC having a total organic matter concentration of about 1400 mg/dm^3 as total COD_{Cr} . The chemical properties of the original potato liquid obtained after mastication and filtration are given in Table 1.

Table 1

Chemical properties of the original solution of potato liquid after mastication and filtration through a 3 μm membrane

Parameter	Value
pH	5.94
EC, mS/cm	0.0518
Total CODCr, mg/dm ³	39550
Soluble CODCr, mg/dm ^{3a}	34850
DOC, mg/dm ^{3a}	11670

^aSoluble CODCr and DOC were measured for the potato liquid after further filtration through membrane filter with the pore size of 0.45 μm .

MFC configuration and operation. A two-chamber MFC designed and fabricated by Feng et al. [12] was used. This reactor had a total volume of 0.25 dm³ for each chamber, and the working volume in the anodic chamber was 0.24 dm³. Carbon felts, each 6 cm long, 4 cm wide and 0.5 cm thick, were used as the anode and cathode. Cation exchange membrane (CEM, Zhejiang Qianqiu Group Co., Ltd., China) was sandwiched between two chambers. The inlet and outlet, sampling, and aeration in the cathodic chamber were also designed in this reactor. Leak proof sealing was used at the joints to prevent penetration of air into the anodic chamber, hence maintaining an anaerobic reaction environment inside the chamber. Titanium wire was used to connect the anode and cathode.

Anaerobic consortia used for inoculation of the anodic chamber were collected from a MFC reactor operated by Hirooka and Ichihashi [15] for treating artificial wastewater containing sodium acetate. Prior to use, the anaerobic consortia (20 cm³) were cultured in a 100 mM phosphate buffer solution (PBS) (Na₂HPO₄·12H₂O 22.2 g/dm³, NaH₂PO₄·2H₂O 5.92 g/dm³, NaCl 5.84 g/dm³, KCl 0.10 g/dm³, NH₄Cl 0.25 g/dm³, pH 7.0, EC 9 mS/cm) containing trace minerals (final concentration of nitrilotriacetic acid (NTA) 19 mg/dm³, MgSO₄ 38 mg/dm³, MnSO₄·H₂O 6.3 mg/dm³, NaCl 13 mg/dm³, FeSO₄·7H₂O 1.3 mg/dm³, CaCl₂·2H₂O 1.3 mg/dm³, CoCl₂·6H₂O 1.3 mg/dm³, ZnCl₂ 1.6 mg/dm³, CuSO₄·5H₂O 0.13 mg/dm³, AlK(SO₄)₂·12H₂O 0.13 mg/dm³, H₃BO₃ 0.13 mg/dm³, Na₂MoO₄ 0.31 mg/dm³, NiCl₂·6H₂O 0.30 mg/dm³, Na₂WO₄·2H₂O 0.31 mg/dm³) and vitamins (final concentration of biotin 2.5 mg/dm³, folic acid 2.5 mg/dm³, pyridoxine HCl: 13 mg/dm³, riboflavin: 6.3 mg/dm³, thiamin 6.3 mg/dm³, nicotinic acid: 6.3 mg/dm³, pantothenic acid: 6.3 mg/dm³, B-12: 0.13 mg/dm³, *p*-aminobenzoic acid 6.3 mg/dm³, thioctic acid: 6.3 mg/dm³). Soon after inoculation, the anodic chamber was operated with sodium acetate until current density reached stable. Subsequently, the anodic chamber was shifted to potato waste prepared by diluting the resulting potato liquid after mastication and filtration through 3 μm membrane filter by about 28 times with PBS. This reactor was operated for 81 days with 100 Ω of external

resistance under the controlled temperature of 30 °C. The pH of the anodic solution was adjusted to 7.0–7.1 every 4–6 days using 1 M NaOH or HCl. The cathodic chamber was fed with the PBS solution and aeration was continuously conducted using an air pump to provide oxygen as the electron acceptor.

Analyses. Voltage was measured every minute by a multimeter with a data acquisition system (midi LOGGER GL200A, Graphtec Corporation, Japan). Current density (I) and power density (P) were calculated based on the equations: $I = V/(RA)$ and $P = V^2/(RA)$, respectively, where A is the surface area of the anode, R is the external resistance and V is the voltage. When current output of the MFC reached a constant value, the polarization behavior was analyzed by adjusting the external resistance from 50 Ω to 4000 Ω .

The total and soluble COD of the liquid samples collected during MFC operation were analyzed by the colorimetric method (DR/890 Colorimeter). The soluble COD was analyzed with liquid samples after filtration through 0.45 μm filters. For the liquid samples after filtration, dissolved organic carbon (DOC) and volatile fatty acids (VFAs) were also analyzed using a total organic carbon analyzer (TOC-V_{CSH}, SHIMADZU, Japan) and a high performance liquid chromatography system (SHIMADZU, Japan), respectively. For VFAs, seven species (citrate, isobutyrate, acetate, propionate, butyrate, valerate and isovalerate) that are generally targeted in investigation of the behavior of biomass (including activated sludge) during fermentation and anaerobic digestion were targeted in this study. EC was analyzed using an EC meter (CM-14P, DKK-TOA Co., Japan).

At the end of the MFC operation, 10 cm³ of the anodic solution and a part of the electrode (1×1×0.5 cm) cut from the whole anode were subjected to the extraction of DNA by the PowerSoil[®] DNA Isolation Kit (MO BIO Laboratories, Inc., Japan), respectively. PCR amplification of 16S rDNA was performed with a real time PCR system (Thermal Cycler Dice, TP800, TAKARA, Japan), for which, GC-341f and 907r were used as primers with a concentration of 20 μM in a mixed volume containing 250 μM Ex Taq DNA polymerase, 2.5 mM dNTPs, 10×Ex Taq buffer, 14.5 μM bovine serum albumin (BSA) and sterilized pure water. The PCR program included denaturation at 95 °C for 5 min, followed by 35 amplification cycles (at 95 °C for 30 s, 57 °C for 30 s and 72 °C for 40 s), and finally by an extension step at 72 °C for 10 min. The obtained PCR products were tested by electrophoresis in 1.2% agarose gel stained with ethidium bromide. DGGE was conducted with 6% (w/v) polyacrylamide gel using the Dcode[™] system (Bio-Rad, USA). The electrophoresis was run for 16 h at 80 V with the denaturant gradient of 30–60%. After electrophoresis, the gels were stained with SYBR[®] Green Nucleic Acid Gel Stain solution (TAKARA) and then visualized with the Gel Doc 2000 System (Bio-Rad, USA). Representative DNA bands were excised from the DGGE gels and then re-amplified by PCR using the same primer set of 341f and 907r. After purification by the EXOSAP IT kit (Affymetrix), the PCR products were sub-

jected to bidirectional sequencing using ABI 3100 Genetic Analyzer (Applied Biosystems, USA). Sequence comparisons were conducted using the BLAST search option in the NCBI nucleotides sequence database, and the taxon of DNA sequences was gained from the Classifier and Sequence Match programs of the Ribosomal Database Project (RDP) II⁶, as reported by Huang et al. [16].

3. RESULTS AND DISCUSSION

3.1. ELECTRICITY GENERATION

Changes in the current density observed during the MFC operation are shown in Fig. 1. Electricity generation was observed from the beginning of the operation. The current density reached its peak value after 4 days and started to decrease. The decreasing trend was stopped and replaced by a dramatic increase after pH adjustment on the day 8. Till day 17, similar decreasing trend repeated for two times and each time recovery was achieved by adjustment of pH for the solution in the anodic chamber. In the followed operation time, although intermittent pH adjustment was also conducted, its effect on the current density became less obvious and a gradually decreasing trend maintained till the end of the whole operation.

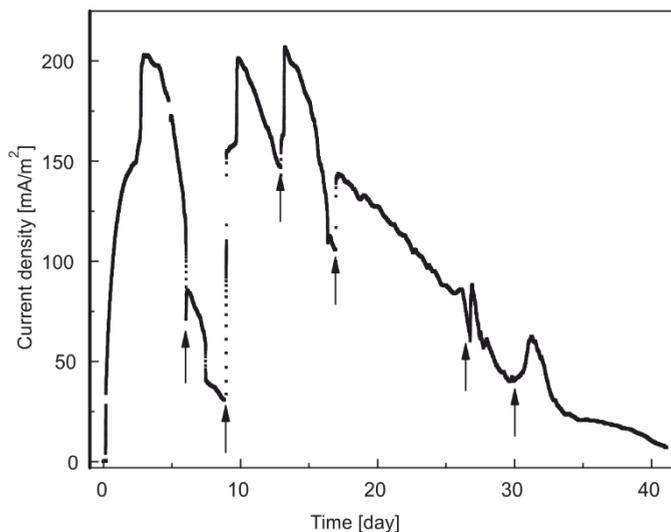


Fig. 1. Time dependence of current density (arrows indicate pH adjustment)

⁶<http://www.rdp.cme.msu.edu>

The decreasing trend observed for three times within the initial 17 days (followed by respective recovery after pH adjustment) was mainly attributed to the rapid hydrolysis of potatoes, which resulted in obvious decrease of pH in the MFC. This was referable since hydrolysis in anaerobic fermentation of organic matter generally leads to formation and accumulation of volatile fatty acids (VFAs) [5] that can be readily used for electricity generation [4]. Our measurement results for VFAs shown later support this explanation. For the trend of gradual decreases of current density observed in the remaining part of operation, however, reductions in the content of biodegradable organic species were probably the major reason behind, which will also be discussed later based on the results of COD and VFAs.

Throughout the operation period, the highest current density was observed after operation for about 14 days with a value of 208 mA/m². This value is similar to the result of Venkata Mohan et al. [5] who conducted an experiment treating the extract from a market available composite vegetable waste and obtained a maximum current density of 215.7 mA/m² with the daily supply of COD for 700 mg/dm³, indicating potato waste alone could also be used as substrate for electricity generation by MFC.

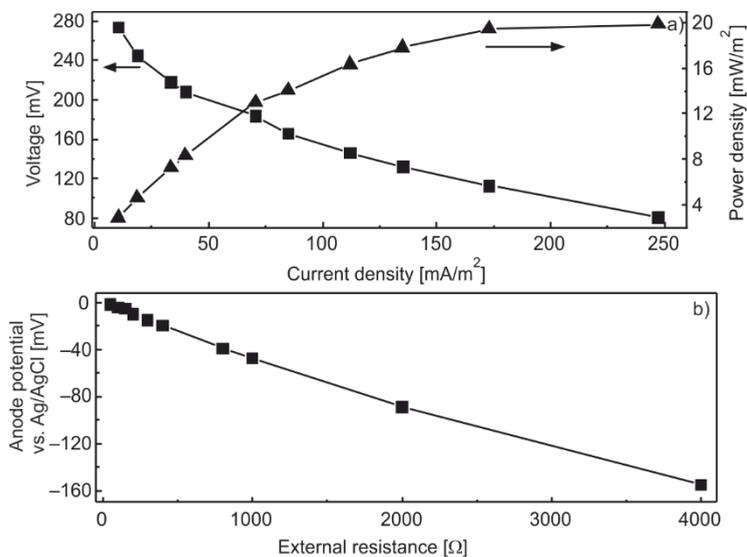


Fig. 2. Polarization measured under various external resistances in the range of 50–4000 Ω when the performance of MFC was stable

Polarization and power density curves are plotted in Fig. 2 to depict the startup of electron discharge and the cell design point where the MFC can be operated effectively. Voltage increased upon the increase of current density, indicating corresponding increase of electron discharge. The polarization curve showed more obvious voltage decreases in the range of current density below 50 mA/m², and then nearly linear decreases

thereafter, suggesting that the activation loss and ohmic loss existed in the MFC. It is reported that the cell design point, i.e., the point of maximum power density, can be used to assess the stability of MFC in its performance [17]. Under the experimental condition of the present study, the cell design point was found at 50Ω with the maximum power density reaching about 20 mW/m^2 and the corresponding current density about 250 mA/m^2 . Our result supports the previous study of Venkata Mohan et al. [5] who reported the maximum power density in the range of $15.4\text{--}26.4 \text{ mW/m}^2$ when treating the extract of the market available composite vegetable waste in a single-chamber MFC.

The internal resistance is one of the parameters significantly affecting the electricity generation efficiency of MFC [18]. In this study, the internal resistance of 99Ω and the electromotive force of 227 mV were estimated from the linear part of the polarization curve, according to the procedure reported elsewhere [1]. Regarding the internal resistance, similar to our result, Feng et al. [12] measured 90Ω of internal resistance in a PPy/AQDS-modified MFC. However, a considerably lower value of 8Ω was reported for a cube-shaped MFC by Logan et al. [18]. The obvious difference may reflect the differences in the biofilm formed on the anode and the solution chemistry (including the organic substrate to be degraded) in the anodic chamber [18], which should be clarified in future studies for more efficient design and operation of MFC with lower internal resistance.

According to Raghavulu et al. [19], the anode potential controls interactions between the biocatalysts and the anode, and thus affecting the kinetics of electron transfer. As shown in Fig. 2b, by varying the external resistance from 50Ω to 4000Ω , the anode potential changed from -1.6 mV to -155.5 mV against a saturated Ag/AgCl reference electrode. This change suggests that the effective electron release from bacteria was achieved [3, 17].

3.2. REMOVAL OF ORGANIC MATTER

In addition to the power generation, an effective degradation of the substrate was also recorded in the MFC. As shown in Fig. 3, the total COD of the anodic solution increased to the highest value of 1860 mg/dm^3 on day 8, was then followed by obvious decrease to about 400 mg/dm^3 on day 30, and slight decrease to 300 mg/dm^3 at the end of the operation. A similar pattern was also observed for soluble COD even if its values being a little bit lower than those of the total COD. The observed increase of COD in the initial 8 days of the operation may indicate that the hydrolysis rate of the masticated potato was higher than the degradation rate inside the anodic chamber. After 30 days, the concentrations of COD available for bacterial use was getting lower, thus causing consistent decreases of current density at a lower level below 50 mW/m^2 . Even if further studies leading to optimization of the treatment system with MFC are necessary, the behavior of COD and the similarity of the time profile of COD with that of the current density discussed earlier (Fig. 1) indicates that MFC can realize simultaneous potato waste removal and electricity generation.

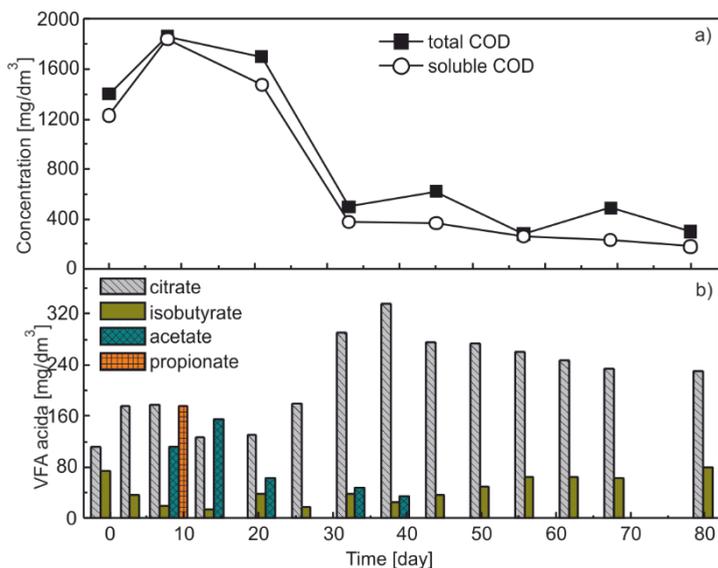


Fig. 3. Dependences of COD (a) and volatile fatty acids (VFAs, b) in the anodic chamber on the operation time

The concentrations of VFAs could reflect the metabolic status of organic matter, as indicated by Zhao et al. [2] who studied the performance of MFC for treatment of cattle dung. In our study, among the targeted seven VFAs (citrate, isobutyrate, acetate, propionate, butyrate, valerate and isovalerate), only citrate, isobutyrate, acetate and propionate were detected. As shown in Fig. 3b, the main VFAs in the anodic solution were citrate and isobutyrate and, although their concentrations fluctuated, both these acids were detected throughout the whole operation. Citrate, as a major component of organic acids in potato tissue [20], reached its highest concentration at 336 mg/dm³. Acetate and propionate were detected within the first 40 days, when current density was recorded (Fig. 1). For propionate, it was only detected on day 10. This may suggest that the degradation rate of this acid species in the experiment system of this study was faster than its generation and accumulation rate. To clarify this, detailed measurement by further shortening the sampling intervals is necessary, which will be conducted in coming studies. Although the net generation and net consumption were not known, the measurement results of both acetate and propionate indicate that hydrolysis occurred in the reactor and that, as organic acids that can be directly used by bacteria [2], their degradation had occurred in the MFC reactor used.

Hydrolysis and degradation of organic matter may cause changes in the ion strength of any treatment systems with bacteria which in turn reflects the extent of the reactions. Higher ion strength was found to be able to facilitate electron transfer [21]. In this study, as a well-used parameter reflecting the ion strength, EC in both the anodic and cathodic chamber solutions were also measured. As shown in Fig. 4a, in the first 30 days, EC in

both chamber solutions revealed increases. The increases of EC in the anodic solution corresponded to the time period (about the first 30 days) when COD available for bacteria use existed as shown in Fig. 3, probably as a result of hydrolysis [22]. The much higher EC values in the cathodic chamber suggested the transfer of some ions produced in the anodic chamber to the cathodic chamber through the CEM.

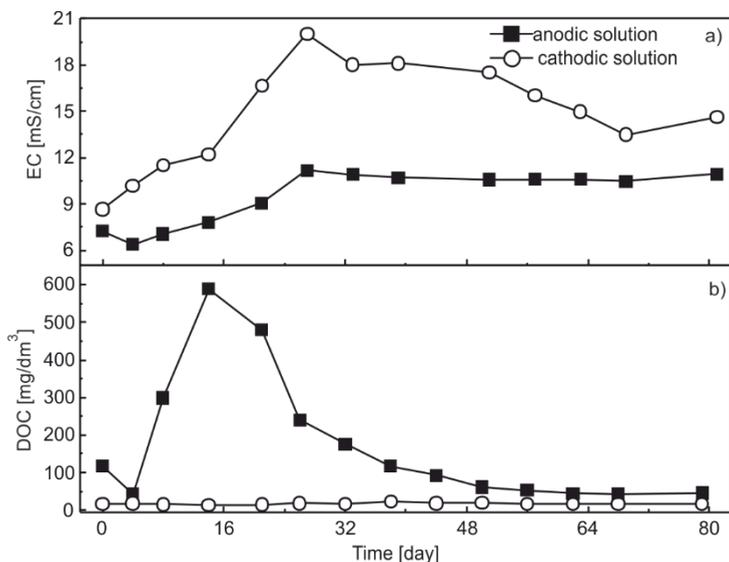


Fig. 4. Dependences of the electrical conductivity (EC) (a) and dissolved organic carbon (DOC, b) in the anode and cathodic chamber solutions on the operation time

Following the trend of COD, DOC in the anodic chamber also changed dramatically, as shown in Fig. 4b. The value of DOC reached its highest value at about 600 mg/dm³ on day 14, then decreased rapidly to about 100 mg/dm³ within about 40 days, and thereafter, slightly decreased to about 50 mg/dm³ at the end. The dramatic increases of DOC in the first 14 days suggested the rapid hydrolysis of fine potato particulates with sizes in the range of 0.45–3 μm existing in the potato liquid; while, the followed rapid decreases reflected effective consumption by bacteria, i.e., the maturity and activity of bacteria populated in the anodic chamber [23]. Different from the EC, the DOC in the cathodic solution was significantly lower than that in the anodic solution, indicating that the transfer of dissolved organic molecules through the CEM used to separate the two chambers was less obvious.

3.3. ANALYSIS OF THE MICROBIAL STRUCTURE

The DGGE images and sequencing results for the microbial community from the anode and anodic solution are given in Fig. 5.

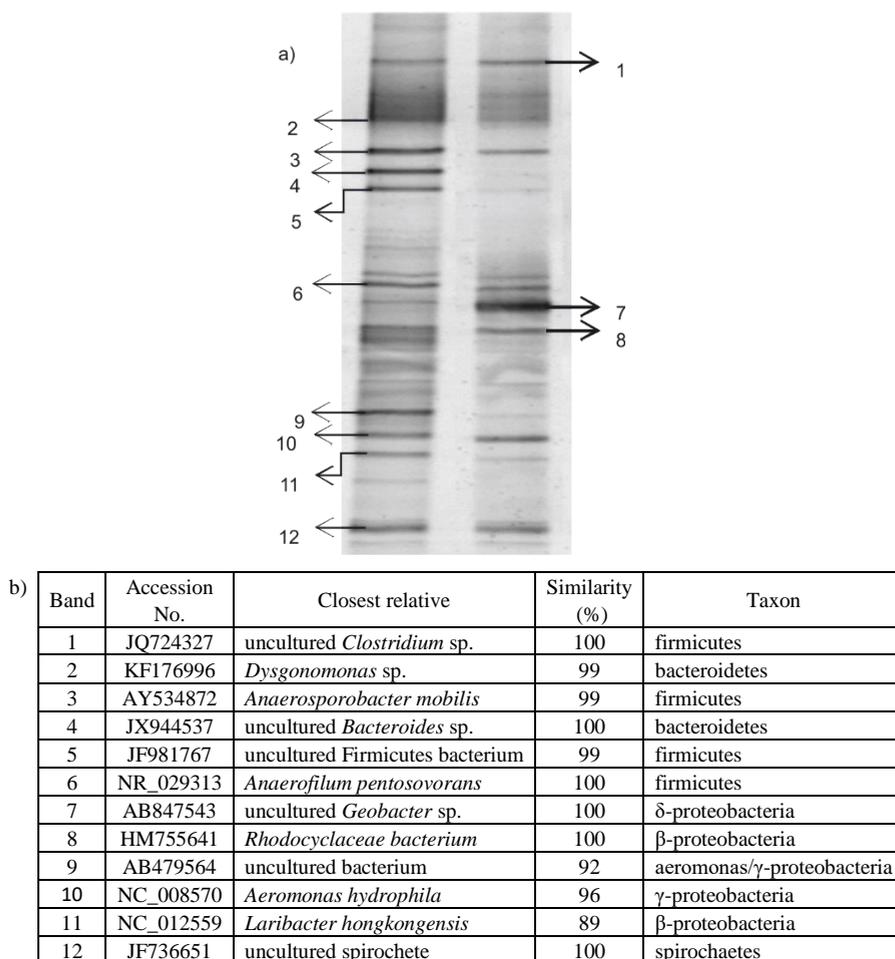


Fig. 5. DGGE images (a) and sequencing data (b) of bacterial 16S rDNA gene fragments from the anode and anodic solution in the MFC. The left lane and right lane in (a) represent the DGGE images of the anodic solution and the anode, respectively

From the DGGE images (Fig. 5a), more bands could be observed in the anodic solution than on the anode, suggesting that more species of bacteria were populated in the solution. Bands 4, 5 and 9 were mainly observed in the anodic solution. From the sequencing results (Fig. 5b), it could be seen that Band 4 affiliates to *Bacteroides*, which can use glycans, protein and animal fats as main energy sources, and can even hydrolyze complex organics. Band 5 affiliates to Firmicutes, which can convert complex organic carbon to simple molecules and scavenge oxygen passing through the separator (such as CEM) in the anodic chamber [24]. Band 9 refers to γ -proteobacteria that have a faster electron collection capability, and were also reported as a good bioindicator for the biodegradation of organic matters in MFC [25]. Bands 1–3, 6, 8, and 10–12 were observed

both on the electrode and in the solution and could be thus considered as common ones in the MFC system. All these bacteria belong to fermentative and electricity generating species. Band 1 affiliates to the *Clostridium* species that can fermentate butyrate and produce hydrogen [11]. Band 2 affiliates to *Dysgonomonas* species that associate with acid production. Band 3 refers to the Firmicutes as Band 5 does, while, Band 6 refers to *Anaerofilum pentosovorans* genus that reduce sulfate to H₂S using polyacrylamide as the sole carbon source [26]. Band 8 refers to the *Rhodocyclaceae* family of β -proteobacteria, belonging to denitrifying rod-shaped species. Band 10 affiliates to the *Aeromonas hydrophila* genus that can reduce ferric iron Fe(III), nitrate and sulfate [10]. Band 11 is represented by the *Neisseriaceae* family of the β -proteobacteria that involve in converting ammonium to nitrite [27], while, Band 12 by *Spirochaetes* that have the function of reductive dechlorination of tetrachloroethylene [28]. Band 7, identified as *Geobacter*, was the predominant band observed on the electrode. *Geobacter* has been well documented in MFC related literature as bacteria that can directly transfer electrons to the electrode [9].

The sequencing analysis results revealed the prominent presence of β -, γ - and δ -proteobacteria and Firmicutes, directly proving that bacterial community that can perform organic matter degradation and electricity generation was formed in the MFC treating potato waste. In addition, *Geobacter* sp., *Aeromonas hydrophila* and *Clostridium butyricum* known to generate electricity in MFCs without the provision of an exogenous mediator [9–11] were also identified. The above sequencing results, together with the results on electricity generation and organic matter removal, indicate that potato waste can be treated by MFC to realize its stabilization and, at the same time, electricity generation.

For potato waste, in addition to its liquid form, which was the treatment target of this study, harvesting, storage, marketing and processing also produce considerably large amounts in solid forms with different sizes. Therefore, investigation of the size effects of potato waste on the performance of MFC is also important, which will be conducted in coming studies.

4. CONCLUSIONS

A two-chamber MFC was operated by supplying potato liquid after mastication of market available fresh potato as the substrate. Through evaluation of electricity generation, organic matter removal and microbial structure, the performance of MFC for treatment of potato waste was investigated. The results demonstrated that using MFC biodegradable organic matter in potato waste can be effectively removed, with the remaining organic constituents at the end of the operation being mainly limited to citrate and isobutyrate. Organic removal was accompanied with electricity generation. Under the treatment condition of this study, the highest current density reached 208 mA/m².

Bacterial structure analysis verified the existence of species involved in hydrolysis, acidogenesis and electricity generation. More species existed in the anodic solution than on the anode, and, in the anodic solution, Proteobacteria, Firmicutes and Bacteroides were dominating phyla, while, on the anode, *Geobacter* species were dominating.

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