

Assessment of Sediment Remediation Potential using Microbial Fuel Cell Technology

S. W. Hong, Y. S. Choi, T. H. Chung, J. H. Song, and H. S. Kim

Abstract—Bio-electrical responses obtained from freshwater sediments by employing microbial fuel cell (MFC) technology were investigated in this experimental study. During the electricity generation, organic matter in the sediment was microbially oxidized under anaerobic conditions with an electrode serving as a terminal electron acceptor. It was found that the sediment organic matter (SOM) associated with electrochemically-active electrodes became more humified, aromatic, and polydispersed, and had a higher average molecular weight, together with the decrease in the quantity of SOM. The alteration of characteristics of the SOM was analogous to that commonly observed in the early stage of SOM diagenetic process (i.e., humification). These findings including an elevation of the sediment redox potential present a possibility of the MFC technology as a new soil/sediment remediation technique based on its potential benefits: non-destructive electricity generation and bioremediation.

Keywords—Anaerobic oxidation, microbial fuel cell, remediation, sediment

I. INTRODUCTION

BIOFUEL cells can be broadly divided into microbial fuel cells (MFCs) and enzymatic fuel cells according to the utilization of either alive microorganisms or isolated redox enzymes as a biocatalyst [1]. The MFC that converts energy stored in the bio-convertible substrate directly to electrical energy has recently received markedly increasing interests.

In the MFC system, electrons are liberated and transferred to the anode by specific bacterial consortium under anaerobic conditions, and flow to the cathode. Subsequently, protons and electrons are consumed at the cathode by the reductive reaction

of oxygen to form water [2]–[4]. Hence, it becomes possible to

generate bio-electricity directly from the chemical energy stored in various types of organic substrates (fuel) using the MFC which provides a double benefit of generating electricity as well as removing organic materials. In view of this electron transfer mechanism, it is well known that *c*-type cytochromes and multi-heme proteins of metal reducing bacteria such as *Geobacter* [5] and *Shewanella* [6] are involved in dissimilatory metal (e.g., iron) reduction. In the same manner, the anode in the MFC can serve as a solid electron acceptor [1].

Soil and/or sediment organic matter (SOM) in the subaqueous environment (i.e. sediment) has been known to be derived from a variety of organic precursors and debris such as planktonic biomasses, land plants, fecal matter, soil leaching, and products of natural burning [6]–[7]. A number of prior studies have demonstrated that the SOM plays a very important role in the ultimate fate of various organic and inorganic contaminants found in the geo-environment.

Recently, the MFC system has been employed in the marine sediments to power electronic devices in remote locations [8]–[9]. The concept of the sediment MFC is fairly straightforward to be implemented: embedding an electrode (anode) in the anaerobic sediment where both of organic substrates and bacteria for the current production are present and connecting it to an electrode (cathode) in the overlying aerobic water. Following the discovery of the sediment MFC, a few efforts have been made to increase power levels, mainly from marine sediments, resulting in boosting the performance in terms of power output. Yet, attempts to study the environmental changes in the bottom sediments during the electricity generation are rare.

The aim of this study was to evaluate the feasibility of the MFC technology as a new remediation method by assessing the generation of electricity and alteration of SOM properties using the sediment MFC systems. It is our expectation that the sediment MFC system provides a potential benefit as a self-sustainable and energy-efficient remediation technology for the contaminated subaqueous environment.

II. MATERIALS AND METHODS

A. Sediment Collection

To assemble sediment MFCs in the laboratory, both anoxic freshwater sediments and surface water samples were collected from two different freshwater environments: one from a

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tributary entering site near the Lake Sihwa ($37^{\circ}11'0''-37^{\circ}23'0''\text{N}$, $126^{\circ}31'0''-127^{\circ}00'0''\text{E}$, hereinafter referred to as Lake Sihwa sediment), the other from the Gongji stream ($37^{\circ}52'0''\text{N}$, $127^{\circ}42'0''\text{E}$, hereinafter referred to as Gongji stream sediment) located in the mid-west and the northeast of Korea, respectively. At both sites, subsurface sediments were collected using a Ponar type grab sampler (2.4 L volume), placed into clean polycarbonate jars (Nalgene, Fisher Scientific, Chicago, IL, USA), and then packed in coolers. Also, the surface water samples were also collected from the both sites.

B. Sediment MFCs

Sediment MFCs having an internal diameter of 100 mm and height of 230 mm were made of flexiglass and placed in a temperature-controlled chamber at 20°C . Using the sediment and water collected from the sites as described above, the anode was buried 4 cm below the sediment surface and the cathode was placed 5 cm above the sediment. Graphite felt (GF series, Electrosynthesis, Amherst, NY, USA) was used for both anode and cathode electrodes ($70 \times 60 \times 6 \text{ mm}^3$). The potential between the anode and cathode across an external load of 10Ω was measured using a multimeter (model 2000, Keithley Instruments, Cleveland, OH, USA) and recorded hourly using a PC-mounted data acquisition board (TestPoint[®], Capital Equipment, Richmond, VA, USA). A schematic diagram of the sediment MFC is provided in Fig. 1. The sediment MFCs were operated in triplicate.

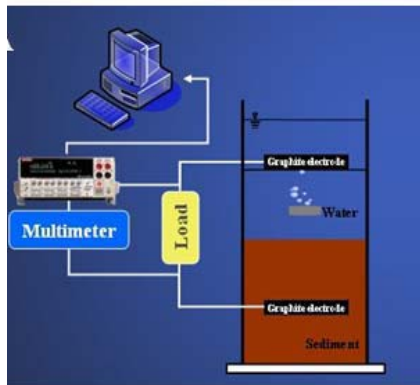


Fig. 1 Schematic diagram of the lab-scale sediment MFC

C. Analyses

The loss on ignition (LOI) was determined gravimetrically by weighing the sample before and after the combustion at 550°C for 4 hours. The readily oxidizable organic matter (ROOM) was determined using the wet oxidation method (cold H_2SO_4) as described by Loring and Rantala [10]. The redox potential was measured with an oxidation-reduction potential (ORP) electrode (9678 BNWP, Thermo Orion, PA, USA).

The sediments incubated under closed- and open-circuit conditions were collected at 143 days of incubation. The samples including the original sediment (hereinafter referred to

as control) were air-dried and ground. Chemical fractionation of sediments based on differences in solubility in alkaline and acid solutions was performed according to the procedure recommended by the International Humic Substances Society [11] with a slight modification.

Organic carbon concentrations in the aqueous samples were determined by a TOC analyzer (TOC-V CPH, Shimadzu, Kyoto, Japan). Spectroscopic absorptivities at 254 and 280 nm were measured to obtain specific ultraviolet absorbance (SUVA) and the ratios of spectroscopic absorptivities at wavelengths of 465 and 665 nm, referred to as E_4/E_6 ratio, were also measured using a UV-visible spectrometer (Genesys 6, Thermo Spectronic, Rochester, NY, USA). The molecular size and the distribution of organic matter were analyzed by high-pressure size exclusion chromatography (HPSEC) using an HPLC system (Agilent LC 1200 series, Agilent Technologies, Inc., Palo Alto, CA, USA) equipped with a Waters Protein Pak 125 SEC column (Waters, Milford, MA, USA) and a photodiode array detector (Agilent Technologies, Inc., Palo Alto, CA, USA).

To determine the Gram reaction of bacteria attached on the anode, they were stained using a LIVE BacLight Bacterial Gram Stain Kit (L-7005, Molecular Probes, Eugene, OR, USA). The images were collected using a LSM 510 confocal laser scanning microscope (CLSM, Carl Zeiss, Jena, Germany) as described by Caldwell et al. [12].

III. RESULTS AND DISCUSSION

A. Electrical Responses from Closed- and Open-Circuit Sediment MFCs

Current productions from the sediment MFCs with two different sources, the Lake Sihwa and Gongji streams, of freshwater sediments were monitored for approximately 5 months (Fig. 2). As shown, the patterns in the current production appeared to be markedly different depending on the sediment sources. On the first day of incubation, the current productions from the sediment MFCs loaded with the Lake Sihwa and Gongji stream sediments were measured as 1.19 and 12.4 mA/m^2 , respectively. In the sediment MFC loaded with the Lake Sihwa sediment, two distinguished current peaks were observed: the first and second appeared on day 11 and 86, respectively. But the current density of the first (17.4 mA/m^2) was approximately $1/2$ of that for the second (35.9 mA/m^2). These trends in the current production demonstrating different peaks over the incubation time were also observed in the sediment MFC for the Gongji stream sediment. In contrast, the magnitude of the first current peak (on day 11) was substantially greater than those of the current peaks appeared on day 26 and between day 45 and 52. The maximum current density of 35.9 mA/m^2 was obtained on day 11 whereas current peaks that appeared in the later stages of the experiment ranged from 15.7 to 20.6 mA/m^2 .

It is possible to explain the occurrence of two current peaks with two potential reasons: biodegradability of the organic matter in the sediment and the enrichment of electrically active

bacteria (EAB) on the electrode, which can be defined as those responsible for the current production in the absence of a mediator [13]. The biodegradable organic matter in the influent wastewater can be classified according to the kinetic criteria as readily and slowly biodegradable variables [14]. Likewise, it is plausible that the SOM employed in our sediment MFCs inherently contained the two types of organic matter. The first current peak in the sediment MFC for the Lake Sihwa sediment may have been attributed to the conversion of readily biodegradable organic matter into electricity. Also, the oxidation of slowly biodegradable organic matter by the EAB enriched on the anode may have produced the second current peak in the sediment MFC. Thus, these observations support that the current production induced by indigenous microorganisms and SOM was dependent on the sediment characteristics: type of organic matter and diversity of microbial populations, essentially responsible for the current production. It seems also reasonable to assume that the Gongji stream sediment contained relatively higher levels of readily biodegradable organic matter and EAB responsible for the electricity generation as compared to the Sihwa Lake sediment. In fact, the sampling site (one of six tributaries flowing into the Sihwa Lake) was heavily contaminated with drainages from steel manufacturers, machine shops, dye houses, and pulp factories [15].

In order to assess the effect of EAB enriched on the electrode when current was produced, the anode used in the closed-circuit sediment MFC of the previous experiment (for the Lake Sihwa sediment) was inserted in a new sediment that was kept in a refrigerator ($>4^{\circ}\text{C}$) until used again. Immediately after connecting the circuit, the current produced from the sediment MFCs equipped with the used anode reached 23.2 mA/m^2 . This value was approximately 20-fold greater than that obtained from the previous experiment when a fresh electrode was inserted into the Sihwa Lake sediment. During the next two weeks, the current production gradually increased and then reached a maximum value of ca. 36 mA/m^2 . The current density ranged from 30 to 35 mA/m^2 with a little fluctuation over the following 7 days. After 28 days of incubation, the current production decreased gradually to ca. 14 mA/m^2 most likely due to the diminution of fuel availability near the anode. It may also have been ascribed to the mass-transfer limitation of organic substrates. In addition, it is perhaps possible that the EAB were enriched well on the anode under closed-circuit conditions, resulting in high levels of current production.

For the comparison of open-circuit potential of each sediment MFC filled with the Lake Sihwa and Gongji stream sediments, the cell voltages monitored over 140 days are also provided in Fig. 3. Within the first 24 hours, the cell voltage of each sediment MFC with the Lake Sihwa and Gongji stream sediments reached 730 and 645 mV vs. standard hydrogen electrode, respectively. Over the course of experimental period, stable open-circuit potentials were observed from both the sediment MFCs as well as slow increases of the potentials to approximately 900 mV. These values indicate that anaerobic conditions were well established near the anode. De

Schamphelaire et al. [16] observed that the anodic potential decreased from -240 mV to -340 mV when the open-circuit potential increased from 695 to 800 mV in the sediment MFCs applied in the rhizosphere of the rice plants.

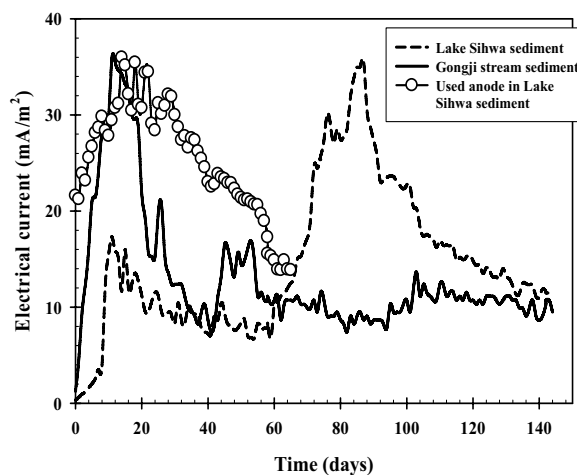


Fig. 2 Current production in the closed-circuit sediment MFCs loaded with the Lake Sihwa and Gongji stream sediment. The enriched anode was buried in the sediment MFC filled with the fresh Lake Sihwa sediment

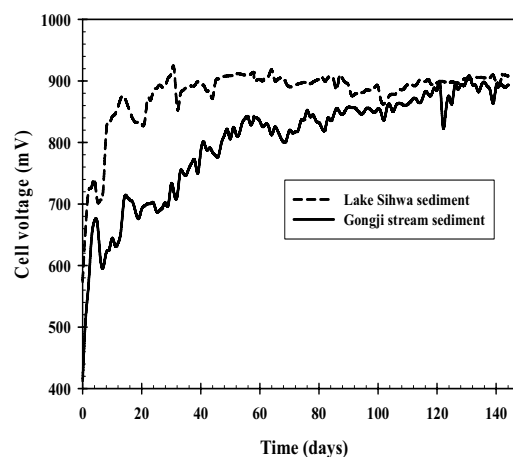


Fig. 3 Voltage generations from the open-circuit sediment MFCs with the Lake Sihwa and Gongji stream sediments

B. Changes of Physicochemical Properties of Sediment under Current Generating Conditions

LOI is a common and widely used method to estimate the organic content of sediment [17]. Under open-circuit conditions, LOI and ROOM decreased from 13.1 to 11.7 % and 6.41 to 5.56 %, respectively. Under closed-circuit conditions, however, there were more decreases in organic matter content than under no-current flowing conditions. After 140 days, the LOI value of the sediment around the anode decreased from 13.1 to 9.32 % and the ROOM value also decreased to 4.20 %. We concluded that the oxidation of organic matter in sediment was accelerated under closed-circuit conditions.

Under closed-circuit conditions, it appeared that organic matter in the sediment was anaerobically oxidized without the production of reduced species such as sulfide compounds or CH_4 . The ORP value of sediments near the anode were $+116.3 \pm 22.9$ mV (mean \pm standard deviation, $n = 8$). In contrast, the value for the open-circuit sediment MFCs was -245.2 ± 18.5 mV (mean \pm standard deviation, $n = 8$). This was due to the change of the redox environment of sediment, from a reduced state to an oxidized state under current generating conditions. Although the amount of sulfide compounds or CH_4 were not directly measured in this experiment, the positive ORP values apparently indicated the anaerobic oxidation of organic matter under closed-circuit conditions because sulfate-reducing and methanogenic conditions exhibit a redox potential less than -200 mV [18].

Table I summarizes variations in properties of the SOM in response to the current production under different conditions including the control sediment (original sediment). According to the previous studies [19]–[20], the E4/E6 ratios and SUVA represent an index of humification of SOM. Increases of SUVA values under closed-circuit conditions have emerged as compared with those under open-circuit conditions. These indicate that the SOM has become more aromatic and condensed [21] during the current production via anaerobic oxidation of organic matter with the anode serving as an electron acceptor as compared to the case of no current production. The SOM modifications concomitant with current production were particularly supported by the moderate decrease of E4/E6 ratios. In general, it has been known that the ratio decreases with increasing molecular size and condensation [19]–[20]. The property changes of the sediment incubated under closed-circuit conditions thus reflect the relative degree of humification which can be partially explained by increasing humic acid fractions (data not presented) and decreasing E4/E6 ratios.

Using an HPSEC system, changes in the physical structure of the SOM concomitant with the electricity generation were observed and compared to those for the control and open-circuit sediments. As presented in Table I, the relative weight-average molecular (M_w) and polydispersity (ρ) were markedly different depending on the incubation condition. Those values were significantly increased along with the current production, suggesting that macromolecules were formed along with electricity generation via anaerobic oxidation of organic matter in the sediment. These were very similar to the results of the prior study [22] demonstrating that macromolecules can be formed by microbial condensation reactions under sulfate-reducing and methanogenic conditions. Thus, our observations for the closed-circuit conditions also support that the anode can be served as an alternative electron acceptor under anaerobic conditions when the circuit is closed.

As depicted in Fig. 4, the relationships between the physical properties and the chemical characteristics of SOM were evaluated by correlating values obtained from the spectroscopic measurements to molecular sizes: e.g., values of SUVA at 254 and 280 nm vs. M_w and E4/E6 ratio vs. M_w . Each

relation exhibited fairly good positive and negative correlations ($0.83 < r^2 < 0.96$), indicating that the larger the molecule in its size, the more humified the OM as demonstrated previously [23].

TABLE I
CHANGES IN PHYSICOCHEMICAL PROPERTIES OF SOM

		Control	Open-circuit	Closed-circuit
SUVA	at 254nm	9.12	10.7	12.8
	(L/mg C-m)	7.53	8.82	10.2
E4/E6 ratio		3.78	3.64	3.15
Molecule size (Da)	M_w^a	13,452	14,245	15,765
	M_n^b	1,663	1,598	1,702
	ρ^c	8.09	8.91	9.26

^aRelative weight-average molecular weight; ^bRelative number-average molecular weight; ^cPolydispersity ($=M_w/M_n$)

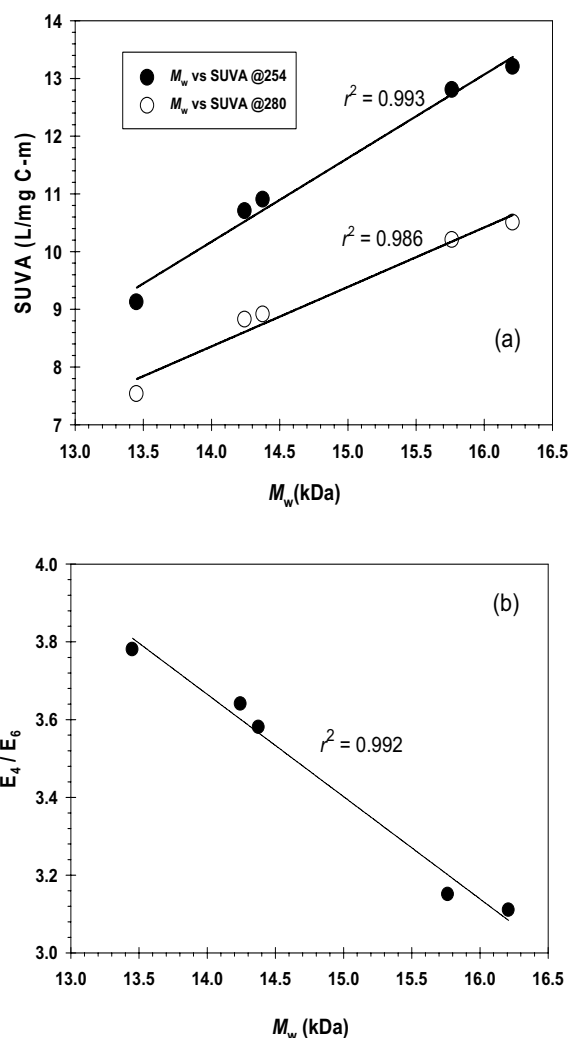


Fig. 4 Relationships between SUVA values at 254 and 280 nm and M_w (a), and between E4/E6 and M_w (b)

C. Electrical Current Responses from Sediment MFCs Amended with External Additives

Until the current production dropped ca. 0.1 mA, the sediment MFCs amended with acetate or acetate + 2-bromoethane-sulfonic acid (BESA) were operated under the same conditions as before for three more consecutive months. Over the course of this period, bio-electrical responses in association with external additives were observed. The injection of additives into the active anode area was made by inserting a piece of polyethylene tube (1.0-mm i.d.) into the graphite felt electrode. In order to quantify the electron recovery as electricity from the additives, the Coulombic efficiency (*CE*), defined as the percentage charge obtained compared to the theoretical charge obtainable from the complete oxidation of the known substrate [24], was calculated as follows:

$$CE = \frac{C_{Ex}}{C_{Th}} \quad (1)$$

where C_{Ex} is the total Coulombs obtained by integrating the current measured at each time interval (*i*) over time using the following equation,

$$C_{Ex} = \left(\sum_{i=1}^T V_i t_i \right) / R \quad (2)$$

where V represents the measured voltage (V) and R is the external resistance (Ω). The theoretical amount of Coulombs (C_{Ex}) that is available from the complete oxidation of acetate can be calculated as follows:

$$C_{Th} = FbMv \quad (3)$$

where F is the Faraday's constant (96,485 C/mol of e^-), b is the mole of electrons produced per mol of substrate (8 mol of e^- /mol of acetate), M is the acetate concentration (mol/L), and v is the liquid volume (L) [25].

Fig. 5 presents the current production from the sediment MFC corresponding to the addition of 15 mM of acetate and 15 mM of acetate plus 10 mM of BESA. The *CE* and the mean value of C_{Th} with standard deviations for the triplicate experiments are also provided in Table II. The reason for adding BESA along with acetate was to assess the effect of methanogens on the *CE* since BESA at 10 mM has been known to inhibit methanogenesis [26]. The maximum current and the duration of current production measured in three individual experiments were similar regardless of additives. The *CE* in responses to the addition of acetate only and acetate plus BESA were calculated as 98.2 and 97.4, respectively. Even though the maximum levels of currents were lower when acetate was added along with BESA than those when acetate only was supplied, the overall levels were similar to each other over the course of reactor run. This was because the duration was

relatively prolonged in case of adding BESA together with acetate. Thus, it appeared that the activity of EAB was marginally affected by the presence of BESA, but further work is required to examine the effect of metabolic inhibition on the electron transfer system of EAB.

Although methane formation was not directly measured from both reactors (closed- and open-circuit sediment MFCs), the apparently high values of *CE* obtained when acetate was added supports that methanogenesis was suppressed in the closed-circuit sediment MFC operated for the prolonged period. This can also be supported by the increased redox potential values for the sediment near the active anode ($> +100$ mV). Therefore, it can be suggested that the suppression of methane production in the sediment MFCs was attributable not to allowing the growth of methanogens but to the increase of the redox potential around the active anode. The previous microcosm studies have demonstrated that dissimilatory iron-reducing bacteria can outcompete both sulfate-reducing bacteria and methanogens for limiting electron donors when bioavailable Fe(III) is available [27]. Likewise, the electrode served as an effective electron acceptor as well as Fe(III) in sediments for the current production in this study.

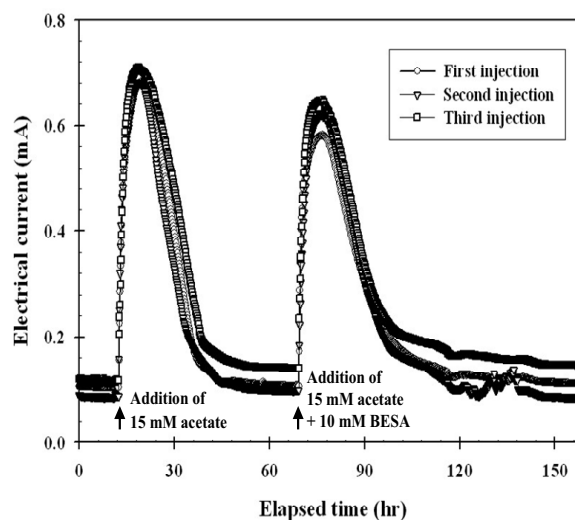


Fig. 5 Current production when acetate (15 mM) only was added or acetate (15 mM) + BESA (10 mM) were added under closed-circuit conditions (three individual experiments for each case)

TABLE II
COMPARISON OF COULOMBIC EFFICIENCIES OBTAINED FROM THE SEDIMENT MFCs AMENDED WITH DIFFERENT ADDITIVES

	15 mM acetate	15 mM acetate + 10 mM BESA
C_{Ex} (C) ^a	35.46 ± 0.67	35.19 ± 0.77
C_{Th} (C)	36.12	36.12
CE (%)	98.2	97.4

^aCurrent levels before adding acetate or acetate + BESA was chosen as the baseline to calculate the mean value (\pm standard deviation, $n=3$).

D. Confocal Microscopic Observations

All electrodes from the sediment MFCs incubated under different conditions (i.e., closed- and open-circuit conditions) were examined by confocal microscopic observations in order to determine the Gram sign in living bacteria attached to the electrodes (Fig. 6). Most microorganisms capable of reducing electrode without the addition of an exogenous electron-carrying mediator such as methylene blue or neutral red have been known as Gram-negative, strictly anaerobic, and nonspore-forming bacteria (e.g., *Shewanella putrefaciens*, *Geobacter metallireducens*, *Rhodospirillum rubrum*, and other humates or Fe(III) reducing bacteria) [2]–[3], [9]. Recently, Milliken and May [28] observed that *Desulfotobacterium hafniense* strain DCB2, the Gram-positive and spore-forming bacterium, can generate electricity in MFCs with the addition of an electron-carrying mediator such as humic acids or humate. Also, prior studies reported that the biofilm and bacterial clumps consist of both Gram-positive and Gram-negative bacteria throughout the electrode surface in the closed-circuit MFCs fed with wastewater collected from a starch-processing plant and artificial wastewater containing the mixture of glucose and glutamate [29]. They attributed to the microbial clumps to the coexistence of fermentators and EAB, respectively, for the fermentation of the complex fuel and the subsequent oxidation of the fermentation products followed by transferring the electrons to the anode.

In Fig. 6 A and C, CLSM reveals that Gram-negative bacteria were dominant throughout the anode surface from the closed-circuit sediment MFCs filled with different sediment sources as observed in the previous study [13]. This observation supports that the EAB in the sediment MFCs were tightly attached to the electrode whereas other fermentative bacteria were loosely attached or present within the sediment matrix in the vicinity the electrode. On the other hand, CLSM observations (Fig. 6 B and D) also demonstrate that Gram-negative and Gram-positive bacteria formed a biofilm-like structure on the anode surface of the open-circuit sediment MFCs, which was relatively less dense than those formed on the active anodes.

IV. CONCLUSION

Given that electrons are liberated from the anaerobic oxidation of organic substrate in MFCs, it can be suggested that the current-harvesting anode can act as an electron acceptor in sediments in a similar manner of other common electron acceptors (e.g., Mn(IV), Fe(III), and sulfate). The main empirical findings derived from this study can be summarized as follows. First, the local redox potential of the sediment in the presence of anode was elevated to positive levels that may have prohibited the substantial growth of methanogens. In addition, the suppression of methane production was indirectly evidenced by high Coulombic efficiencies (> 97%) in response to the external fuel addition (acetate). Second,

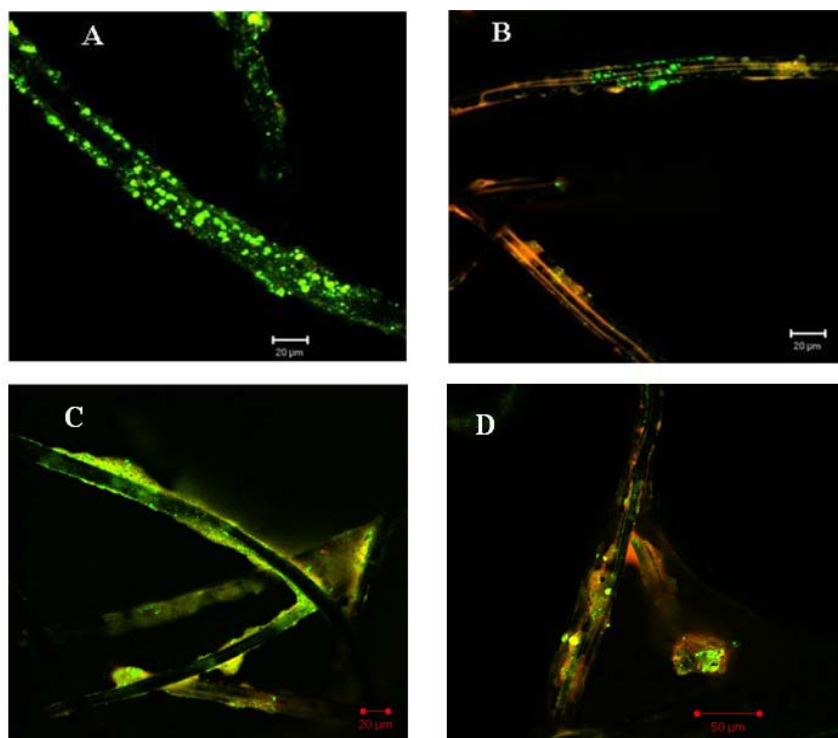


Fig.6 CLSM of the biofilm developed on the anode in closed-(A and C) and open-(B and D) circuit sediment MFCs operated for approximately 5 months (A and B for Lake Sihwa sediment; C and D for Gongji steam sediment; Red: Gram positive; green: Gram negative)

humification-like process was comparably more stimulated under closed-circuit conditions compared to no current conditions. These are new findings associated with electricity generation and may present a potential for the energy-efficient remediation, monitoring, and/or management of geo-environment if further efforts are made.

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