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Biologically activated graphite fiber electrode for autotrophic acetate production from CO_2 in a bioelectrochemical system

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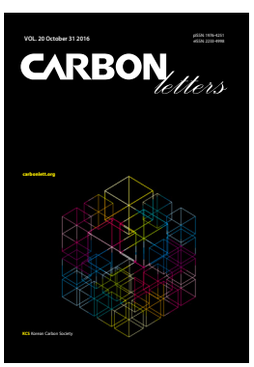

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Recently, microbial electrosynthesis (MESs) has been highlighted for the purpose of biological CO_2 reduction with simultaneous production of intermediates and value-added chemicals. The bioelectrochemical system (BES), which employs microorganisms and a bacterial community as a biocatalyst, has been developed to convert CO_2, a greenhouse gas, into liquid biofuels, such as ethanol and butanol, as well as platform chemicals [1]. Several bacterial species, called cathodophilic microorganisms (e.g., *Sporomusa ovata* and *Clostridium ljungdahlii*) were reported to interact with a carbon electrode by accepting electrons supplied externally from a power supply [2-4]. Through this process, oxidized chemical molecules, such as CO_2, can be converted to more reduced products, such as acetate and ethanol [4,5]. Since the first report of MESs with *S. ovata* [3,4], performance has been improved by efforts to optimize the reactor design, regulate the applied potential, and improve the bacterial enrichment method [6-8]. On the other hand, the interaction between microorganism and carbon materials is still unknown, which is the main factor limiting further improvement of the performance of the MES process. For example, insufficient information about microbe-carbon interactions is delaying the advance of the process significantly when the input potential is <−410 mV vs standard hydrogen electrode (SHE), which is the theoretical minimum potential for hydrogen production [9].

Carbon has been recognized as the best material for fuel cell applications owing to its good electrical conductivity, its potential for the impregnation of catalysts, and its cost effectiveness [10,11]. For the same reasons, a range of morphologies of carbon materials have been utilized as anode and cathode electrodes in BES in the form of carbon paper, cloth, graphite rod, and felt in BES systems [12]. The porous structure of carbon can also provide sufficient surface area for bacterial attachment, allowing the establishment of an electron transport system to the electrode, and good stability (i.e., resistant to oxidation and reduction). Modified carbon electrodes with chitosan and cyanuric chloride improved their electrosynthetic performance more than 6-fold compared to an untreated carbon electrode [12]. The excellent formation of biofilm on the carbon electrode, acting as a catalyst for MESs, can provide a novel application platform of carbon materials for useful chemical production and reduction of greenhouse gas with biotechnology.

The electrochemical properties of microorganisms on the carbon electrode were investigated by cyclic voltammetry (CV), linear sweep voltammetry, and electrochemical impedance spectroscopy (EIS) to elucidate the electron transfer mechanisms between the bacteria and the electrode surface [13,14]. These analyses provided information on how electro-active bacteria interact with the carbon electrode, and the appropriate range of applied potentials in BES [6,8]. For the first demonstration of electromethanogenesis, methane was produced from CO_2 at applied potentials below −700 mV vs Ag/AgCl. Biofilm formation on the carbon electrode was reported to increase current generation and improve methane production [15]. Patil et al. [8] showed that galvanostatic operation facilitated biofilm formation on the electrode, which was demonstrated by CV and confocal microscopy, indicating that 58% of the electrons were recovered in acetate. EIS has been used to examine the electron transfer mechanisms in BESs [13]. The charge transfer resistance was decreased significantly with the development of the biofilm community on the carbon electrode [16].

This study investigated acetogenic biofilm formation on a carbon electrode with the production of acetate in BES. The coulombic recovery estimated with the acclimated biocathode was <−1.11 V vs Ag/AgCl. Through various technical analysis methods, such as EIS, CV, and scanning electron microscope (SEM), the biologically modified carbon electrode was found to be an alternative way to achieve carbon dioxide capture and the simultaneous production of intermediate chemicals, as well as an alternative method for storage of electrical energy.

Anaerobic digester sludge collected from the secondary digester of the Suyoung Wastewater Treatment Plant (Busan, Korea) was used as the inoculum for start-up of the MESs in BES. The collected sludge was washed three times in phosphate buffered saline (PBS buffer) before inoculation to remove organic compounds and then stored in a refrigerator until use. The bacterial growth medium used contains the following components (per liter of distilled water): 1.5 g KH_2PO_4, 2.9 g K_2HPO_4, 2.0 g $NaHCO_3$, 0.5 g NH_4Cl, 0.09 g $MgCl_2{\cdot}6H_2O$, and 0.0225 g $CaCl_2{\cdot}2H_2O$. It was supplemented with 10 mL Wolfe's mineral solution and 10 mL Wolfe's vitamin solution. Next, 5 mM of sodium 2-bromoethanesulfonate was added to inhibit methanogenic activity and enhance autotrophic acetate production [6]. All experiments were carried out at 30°C.

A H-type BES was constructed from two customized glass bottles (325 mL capacity; Corning, NY, USA) connected by a glass tube containing a proton exchange membrane (PEM; Nafion 117, Dupont, Wilmington, DE, USA) (inner diameter, 2.4 cm). Each bottle has two sampling ports for the gas and liquid. The two chambers and a PEM were held by a clamp in the middle of the tube [17]. The cathode was graphite carbon felt (4 × 5 × 0.5 cm; GF-S6-06, Electrolytica Inc., Amherst, NY, USA), which was treated with 1 M HCl for 1 h and washed with deionized water. The anode electrode was a graphite rod (CeraMaterials, Port Jervis, NY, USA). Both carbon electrodes were connected to titanium wire (Sigma-Aldrich) and sealed with epoxy glue. The Ag/AgCl reference electrode (RE-5B; BASi, West Lafayette, IN, USA) was placed in the cathode chamber. Each chamber was purged with ultrahigh purity nitrogen gas (5 ppm impurity) for 15 min to remove trace oxygen and to maintain anoxic conditions before inoculation and start-up. Subsequently, 20% CO_2 mixed gas (N_2:CO_2 = 80:20) was provided continuously into the cathode chamber through a needle valve (Swagelok, Solon, OH, USA) at a 10 mL/min flow rate. A potential of −1.11 V vs Ag/AgCl on the cathode electrode for the start-up and main experiment, was continuously applied using a potentiostat (VersaSTAT 3; Princeton Applied Research, Oak Ridge, TN, USA) to drive the reduction reaction of the CO_2. All potentials reported are referenced to the Ag/AgCl reference electrode.

A 1 mL liquid sample was collected every day and analyzed by a gas chromatograph (7890B; Agilent, Santa Clara, CA, USA) equipped with a flame ionization detector and a HP-FFAP column (Agilent). It was used to quantify the alcohols, acetate, propionate, butyrate, ethanol, and volatile fatty acids produced. To investigate the redox activity on the cathode carbon electrode surface, CV was used with a range of −1.1 to +0.2 V vs Ag/AgCl at a scan rate of 1 mV/s. The effect of the electron acceptor presence with and without a biocatalyst was investigated by CV. EIS was analyzed by sweeping the frequency between 10 mHz and 100 kHz with a 10 mV amplitude. More detailed morphological changes on the surface of the carbon electrode were investigated using a SEM (SEC SNE-3000 MB; Suwon, Korea) with a 30 kV acceleration voltage at room temperature. The samples were fixed overnight in 2.5% glutaraldehyde in 10mM HEPES (pH 7.4), dehydrated in a graded series of ethanol solutions (30%–100%), and dried with bis(trimethylsilyl) amine (Sigma Aldrich). Finally, the samples were mounted on aluminum stubs, sputtered with gold, and examined using SEM.

The BES was inoculated with digester sludge and operated for two weeks under −1.11 V vs Ag/AgCl. The electrosynthetic acetogenic microorganisms contained in the sludge were acclimated on the electrode during this period (data not shown). Prior to the main experiment, 90% of the liquid phase was replaced with fresh medium after 2 weeks to selectively adapt the autotrophic acetogens on the cathode electrode and remove the acetate consuming bacteria in the liquid phase. During this process, only the electrode associated and/or applied potential preferable bacteria on the electrode (i.e., acclimated biocathode) remained in the BES reactor. The acclimated biocathode began to consume current at approximately −38 mA (i.e., reductive current) on 35 h from the beginning of the main experiment, which shifted progressively to a more positive current, and then to less than a applied potential of −1.11 V vs Ag/AgCl (Fig. 1a). Acetate was produced simultaneously, from CO_2 in the cathode

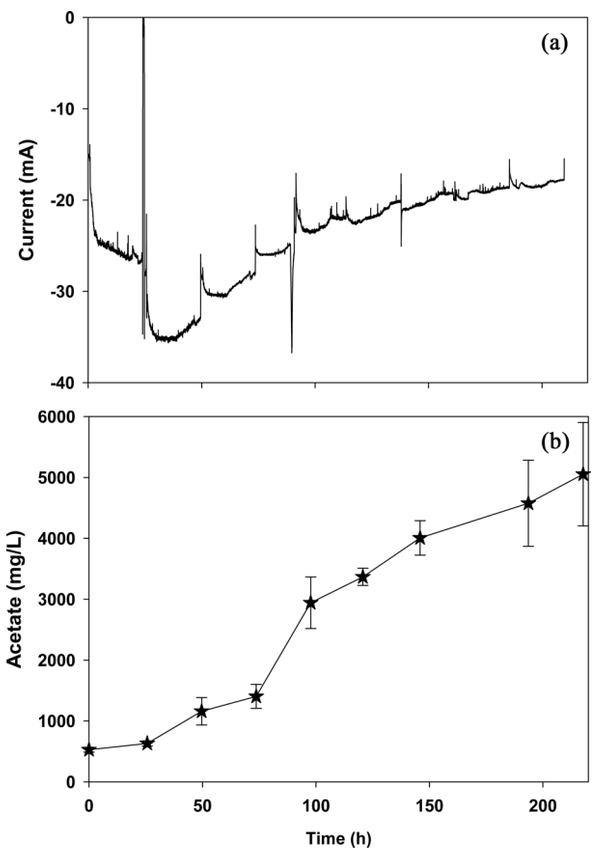

Fig. 1. Current consumption profile at the biocathode in the bioelectrochemical system during the main electrosynthetic process (a), and production of acetate (b).

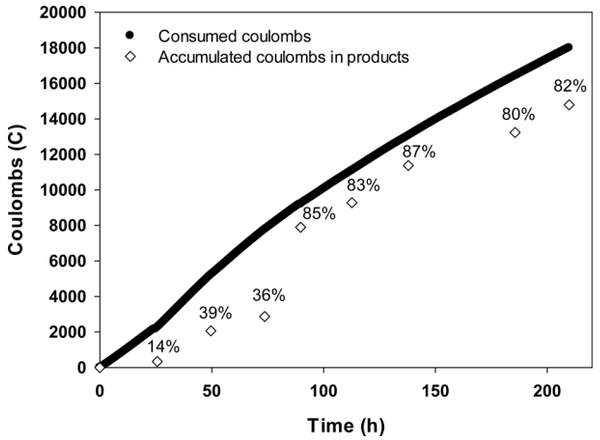

Fig. 2. Coulombic efficiency of autotrophic CO_2 reduction to acetate: the circle indicates the consumed coulombs and the open diamonds are the accumulated coulombs in the products. The estimated coulombic efficiencies are presented on the open diamonds.

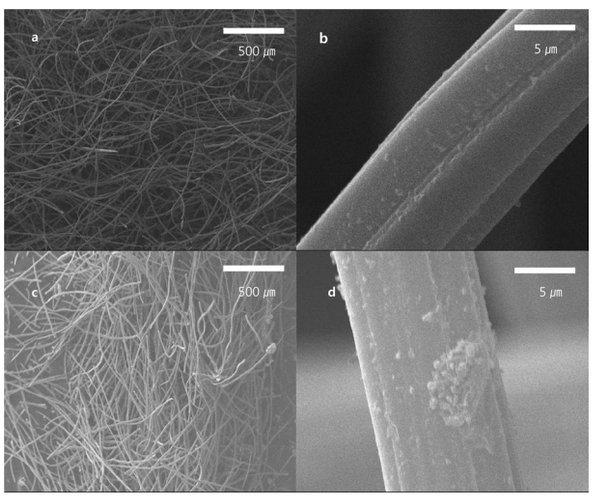

Fig. 3. Scanning electron microscope image (a, b) before inoculation (c, d) after cultivation with sludge.

chamber (Fig. 1b).

The estimated coulombic efficiency (CE) indicates the level of electrons recovered to acetate from the current provided by the potentiostat. The CE was calculated using following equation:

$$2CO_2 + 8H^+ + 8e^- \rightarrow CH_3COOH + 2H_2O$$

$$\mathrm{CE}(\%) = \frac{nFm}{\int_0^t Idt} \times 100\,\%$$

where n denotes the moles of recovered electrons in the products, F is Faraday's constant (96,485 C/mole of e^-), m is the acetate produced during operation from 0 to t, and I is the current recorded by the potentiostat.

The CE remained relatively low (approximately 40%) in the initial period; however, it increased to over 80% at the final

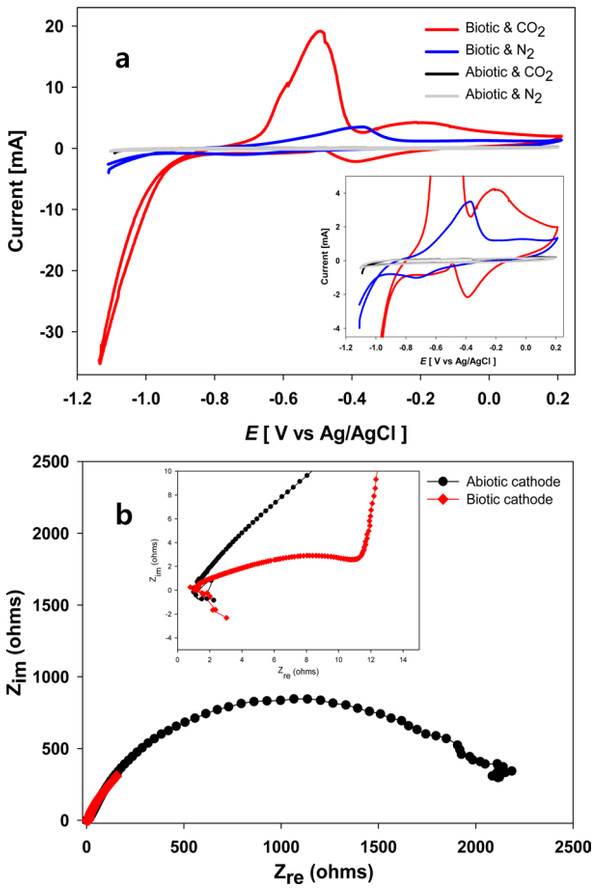

Fig. 4. Cyclic voltammetry analysis of the effect of the electron acceptor presence with and without bacteria (a), electrochemical impedance spectroscopy analysis with the biotic and abiotic cathode (b). The frequency range was between 10 mHz and 100 kHz, with a 10 mV amplitude.

stage, indicating that the microorganisms attached to the cathode had adapted to the electrophilic autotrophic conditions (Fig. 2). The unrecovered coulombs in the initial period appear to have accumulated in precursors such as formate and hydrogen; then some of the precursors might have been converted to acetate by electrosynthetic CO_2 conversion in the later phase [18]. The estimated acetate production rate was 561 mg/L/d, which is relatively higher than the previously reported autotrophic CO_2 reduction [19-21].

The attached electrochemically active bacteria on the carbon electrode were examined by SEM (Fig. 3). The microbial community and cell lumps were observed on the fiber of the graphite electrode (Fig. 3c and d). These colonized cells on the electrode interact with the electrode by gaining electrons from the external power supply and utilizing them to reduce CO_2 via a form of the bacterial energy metabolism [22].

The electrochemical properties of the biologically activated cathode electrode (biocathode), which performed electrosynthetic CO_2 reduction, were compared with those of an abiotic cathode electrode (i.e., a control without bacteria) using CV (Fig. 4a). The biocathode showed a significantly

higher redox peak at −0.5 and −0.4 V on the forward and reverse scans, respectively. The reductive (cathodic) current increased dramatically with a lower potential below −0.9 V vs Ag/AgCl. The redox peaks decreased significantly when N_2 gas was provided instead of CO_2. These results suggest that the bacteria attached to the cathode actively utilize the supplied electrons from the potentiostat for CO_2 uptake as a substrate in BES. On the other hand, the abiotic control did not show any appreciable redox peaks, and there was no difference between the CO_2 and N_2 conditions. The bacteria on the graphite electrode properly activated the electrode, and acted as an electron consumer to produce acetate from CO_2.

Fig. 4b presents the AC impedance spectra of the biotic and abiotic electrode [13]. From the Nyquist plot, the charge transfer resistance decreased in the biocathode compared to its abiotic counterpart. The estimated charge transfer resistance in the biocathode and abiotic control were 7.52 and 1047.8, respectively. The EIS result supports the hypothesis that the acetogenic bacteria biologically activated the cathode, and simultaneously reduced the charge transfer resistance at the interface between electrode and bacteria.

Further study of an enrichment strategy of autotrophic acetate-producing bacteria on the electrode, optimization of the electrode and operating condition, and the design of a better reactor system for gaseous substrates, such as CO_2, should be investigated to achieve a scaled-up system and a sustainable carbon capture process.

The goal of this study was investigation of the electrochemical characteristics of a biologically activated carbon electrode for MESs. SEM showed that autotrophic acetate producing bacteria had clearly colonized and acclimated to the electrode. The performance of the established biocathode produced an acetate production rate and total coulombic recovery of 561 mg/L/d and 82%, respectively. No significant electrochemical redox activity was observed in the abiotic electrode, whereas the biocathode showed appreciable redox peaks by CV. The presence and absence of CO_2, an electron acceptor, clearly changed the electrochemical properties of the electrode, which indicate that the bacteria use CO_2 as a substrate to produce acetate. The AC impedance spectra showed that the biocathode decreased the charge transfer resistance at the interface and activated electrosynthetic acetate production from CO_2.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgements

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References

[1] Kondaveeti S, Min B. Bioelectrochemical reduction of volatile fatty acids in anaerobic digestion effluent for the production of biofuels. Water Res, **87**, 137 (2015). http://dx.doi.org/10.1016/j.watres.2015.09.011.

[2] Tremblay PL, Höglund D, Koza A, Bonde I, Zhang T. Adaptation of the autotrophic acetogen Sporomusa ovata to methanol accelerates the conversion of CO_2 to organic products. Sci Rep, **5**, 16168 (2015). http://dx.doi.org/10.1038/srep16168.

[3] Nevin KP, Woodard TL, Franks AE, Summers ZM, Lovley DR. Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. MBio, **1**, e00103 (2010). http://dx.doi.org/10.1128/mBio.00103-10.

[4] Nevin KP, Hensley SA, Franks AE, Summers ZM, Ou J, Woodard TL, Snoeyenbos-West OL, Lovley DR. Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms. Appl Environ Microbiol, **77**, 2882 (2011). http://dx.doi.org/10.1128/AEM.02642-10.

[5] Steinbusch KJJ, Hamelers HVM, Schaap JD, Kampman C, Buisman CJ. Bioelectrochemical ethanol production through mediated acetate reduction by mixed cultures. Environ Sci Technol, **44**, 513 (2010). http://dx.doi.org/10.1021/es902371e.

[6] Marshall CW, Ross DE, Fichot EB, Norman RS, May HD. Electrosynthesis of commodity chemicals by an autotrophic microbial community. Appl Environ Microbiol, **78**, 8412 (2012). http://dx.doi.org/10.1128/AEM.02401-12.

[7] Zaybak Z, Pisciotta JM, Tokash JC, Logan BE. Enhanced start-up of anaerobic facultatively autotrophic biocathodes in bioelectrochemical systems. J Biotechnol, **168**, 478 (2013). http://dx.doi.org/10.1016/j.jbiotec.2013.10.001.

[8] Patil SA, Arends JBA, Vanwonterghem I, van Meerbergen J, Guo K, Tyson GW, Rabaey K. Selective enrichment establishes a stable performing community for microbial electrosynthesis of acetate from CO_2. Environ Sci Technol, **49**, 8833 (2015). http://dx.doi.org/10.1021/es506149d.

[9] Kracke F, Vassilev I, Krömer JO. Microbial electron transport and energy conservation: the foundation for optimizing bioelectrochemical systems. Front Microbiol, **6**, 575 (2015). http://dx.doi.org/10.3389/fmicb.2015.00575.

[10] Yuan H, Meng LY, Park SJ. KOH-activated graphite nanofibers as CO_2 adsorbents. Carbon Lett, **19**, 99 (2016). http://dx.doi.org/10.5714/cl.2016.19.099.

[11] Kim KY, Jung Y, Kim S. Study on urea precursor effect on the electroactivities of nitrogen-doped graphene nanosheets electrodes for lithium cells. Carbon Lett, **19**, 40 (2016). http://dx.doi.org/10.5714/CL.2016.19.040.

[12] Zhang T, Nie H, Bain TS, Lu H, Cui M, Snoeyenbos-West OL, Franks AE, Nevin KP, Russell TP, Lovley DR. Improved cathode materials for microbial electrosynthesis. Energy Environ Sci, **6**, 217 (2013). http://dx.doi.org/10.1039/c2ee23350a.

[13] Sekar N, Ramasamy RP. Electrochemical impedance spectroscopy for microbial fuel cell characterization. J Microb Biochem Technol, **S6**, 004, (2013). http://dx.doi.org/10.4172/1948-5948.s6-004.

[14] Harnisch F, Freguia S. A basic tutorial on cyclic voltammetry for the investigation of electroactive microbial biofilms. Chem Asian J, **7**, 466 (2012). http://dx.doi.org/10.1002/asia.201100740.

[15] Cheng S, Xing D, Call DF, Logan BE. Direct biological conversion of electrical current into methane by electromethanogenesis. Environ Sci Technol, **43**, 3953 (2009). http://dx.doi.org/10.1021/es803531g.

[16] Borole AP, Aaron D, Hamilton CY, Tsouris C. Understanding long-term changes in microbial fuel cell performance using electrochemical impedance spectroscopy. Environ Sci Technol, **44**, 2740 (2010). http://dx.doi.org/10.1021/es9032937.

[17] Kim JR, Cheng S, Oh SE, Logan BE. Power generation using different cation, anion, and ultrafiltration membranes in microbial fuel cells. Environ Sci Technol, **41**, 1004 (2007). http://dx.doi.org/10.1021/es062202m.

[18] LaBelle EV, Marshall CW, Gilbert JA, May HD. Influence of acidic pH on hydrogen and acetate production by an electrosynthetic microbiome. PLoS ONE, **9**, e109935 (2014). http://dx.doi.org/10.1371/journal.pone.0109935.

[19] Premier GC, Kim JR, Massanet-Nicolau J, Kyazze G, Esteves SRR, Penumathsa BKV, Rodríguez J, Maddy J, Dinsdale RM, Guwy AJ. Integration of biohydrogen, biomethane and bioelectrochemical systems. Renew Energy, **49**, 188 (2013). http://dx.doi.org/10.1016/j.renene.2012.01.035.

[20] Jourdin L, Freguia S, Donose BC, Chen J, Wallace GG, Keller J, Flexer V. A novel carbon nanotube modified scaffold as an efficient biocathode material for improved microbial electrosynthesis. J Mater Chem A, **2**, 13093 (2014). http://dx.doi.org/10.1039/c4ta03101f.

[21] Batlle-Vilanova P, Puig S, Gonzalez-Olmos R, Balaguer MD, Colprim J. Continuous acetate production through microbial electrosynthesis from CO_2 with microbial mixed culture. J Chem Technol Biotechnol, **91**, 921 (2015). http://dx.doi.org/10.1002/jctb.4657.

[22] Fu Q, Kuramochi Y, Fukushima N, Maeda H, Sato K, Kobayashi H. Bioelectrochemical analyses of the development of a thermophilic biocathode catalyzing electromethanogenesis. Environ Sci Technol, **49**, 1225 (2015). http://dx.doi.org/10.1021/es5052233.