Biologically activated graphite fiber electrode for autotrophic acetate production from CO$_2$ in a bioelectrochemical system

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Recently, microbial electrolysis (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 microorganisms 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 <−1.1 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 KH₂PO₄, 2.9 g K₂HPO₄, 2.0 g NaHCO₃, 0.09 g MgCl₂·6H₂O, and 0.0225 g CaCl₂·2H₂O. 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 (Cera Materials, Port Jervis, NY, USA), which was treated with 1 M HCl for 1 h and washed with deionized water. 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% CO₂ mixed gas (N₂:CO₂ = 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 CO₂. 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 10 mM 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 acetonogens 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 CO₂ in the cathode

![Fig. 1](http://carbonlett.org)
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 CO$_2$ reduction activity compared to the abiotic cathode.
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 N₂ gas was provided instead of CO₂. These results suggest that the bacteria attached to the cathode actively utilize the supplied electrons from the potentiostat for CO₂ 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 CO₂ and N₂ conditions. The bacteria on the graphite electrode properly activated the electrode, and acted as an electron consumer to produce acetate from CO₂.

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 bio cathode 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 CO₂, 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 MEAs. SEM showed that autotrophic acetate producing bacteria had clearly colonized and acclimated to the graphite electrode, whereas the biocathode showed appreciable redox electrochemical redox activity was observed in the abiotic counterpart. 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 CO₂, should be investigated to achieve a scaled-up system and a sustainable carbon capture process.

Conflict of Interest

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

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