

Production of Acetate from Carbon Dioxide in Bioelectrochemical Systems Based on Autotrophic Mixed Culture

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Bioelectrochemical systems (BESs) have been suggested as a new technology for wastewater treatment while accomplishing energy and chemical generation. This study describes the performance of BESs based on mixed culture that are capable of reducing carbon dioxide to acetate. The cathode potential was a critical factor that affected the performance of the BESs. The rate of acetate production increased as the electrode potential became more negative, from 0.38 mM d⁻¹ (-900 mV vs. Ag/AgCl) to 2.35 mM d⁻¹ (-1,100 mV), while the electron recovery efficiency of carbon dioxide reduction to acetate increased from 53.6% to 89.5%. The microbial population was dominated by relatives of *Acetobacterium woodii* when a methanogenic inhibitor was added to the BESs initially.

Keywords: Bioelectrochemical systems, carbon dioxide, acetate, biocathode

Introduction

Bioelectrochemical systems (BESs) such as microbial electrolysis cells have been suggested as a new technology for wastewater treatment while accomplishing energy and chemical generation [21, 22]. The reduction of carbon dioxide in BESs may be a promising way to simultaneously reduce carbon dioxide emissions and generate fuels or other organic compounds [3, 9, 11, 13, 18, 19, 28], which is significant in terms of the environment, energy, and resources.

Using pure culture, it was observed in previous studies that several acetogenic bacteria have the ability to accept electrons directly from an electrode, a process termed microbial electrosynthesis, to reduce carbon dioxide to acetate. Interestingly, the acetogen *Acetobacterium woodii* was unable to consume current [18, 19]. Methanogenic and acetogenic bacteria usually share the same ecosystem [1, 15]. The possibility of simultaneously producing methane and acetate from carbon dioxide in BESs based on mixed cultures has been reported only very recently, both by Marshall *et al.* [14] and in our previous study [8]. Marshall *et al.* [14] observed that *Methanobacterium* spp. and *Acetobacterium*

spp. are the most abundant microorganisms for methane and acetate production, respectively. The previous study suggested that the metabolic pathway and end-products of mixed culture are highly dependent on the set cathode potentials. However, methane has a much higher global warming potential than carbon dioxide [5]. A promising long-term objective is production of valuable chemicals from carbon dioxide.

The aim of this study was to gain a deeper understanding of the performance of BESs for acetate production from carbon dioxide based on mixed culture. To accomplish this objective, bioelectrochemical experiments were performed in the presence of 2-bromoethanesulfonic acid as an inhibitor of methanogenesis [23]. Setting electrode potentials is a useful approach to controlling the performance of BESs [6, 12, 26]. The effect of set cathode potentials on the performance of BESs for the production of acetate from carbon dioxide was thus also evaluated in this study.

Materials and Methods

Bioelectrochemical Cell Setup

In this study, two-chamber BESs were constructed based on

that previously described in [24]. The anode and cathode chambers were of equal volume (150 ml) and were separated by a cation exchange membrane (CEM; CMI7000). The anode electrode was a titanium plate and the cathode electrode was carbon felt. The electrodes were of the same geometric dimensions (4.5 cm × 4.5 cm). The reference electrode was Ag/AgCl (sat. KCl, 197 mV vs. SHE). Throughout this paper, all voltages are reported with respect to Ag/AgCl. Electrochemical potentiostatic measurements and monitoring were performed with EC550 (Wuhan, China).

Enrichment of Electroactive Mixed Culture Biofilm

Prior to the experiments, electrochemical active bacteria were inoculated from activated sludge obtained from a local sewage treatment plant (Chengdu, China). Ten milliliters of activated sludge and 130 ml of medium were introduced into the cathode chamber. The medium contained the following (per liter of distilled water): 10.92 g Na₂HPO₄·12H₂O, 3.04 g KH₂PO₄, 2.25 g NaCl, 0.5 g MgSO₄·7H₂O, 0.5 g NH₄Cl, 0.25 g CaCl₂, 4.0 g NaHCO₃, 1.0 g yeast extract, 2.11 g 2-bromoethanesulfonic acid, 10 ml of trace metal solution [17], and 10 ml of vitamin solution [18]. To the anode chamber was added 140 ml of the same medium omitting yeast extract and it was flushed with ultrapure CO₂ (purity > 99.999%). The cathode chamber was flushed with a H₂-containing gas mixture (CO₂/H₂ = 1:2). The process consisted of 8 cycles, each lasting 5 days. At the end of a single cycle, 80% of the medium was replaced with fresh medium and the gas phase was switched to process the next cycle. At the start of cycle 4, yeast extract was omitted in the medium in the cathode chambers. At the start of cycle 6, the H₂-containing gas mixture was switched to ultrapure CO₂ in the cathode chambers. Throughout this biofilm enrichment process, the cathode potential was set at -900 mV to serve as another source of electrons.

Bioelectrochemical Experiments

For the bioelectrochemical experiments, the bioelectrochemical cell was connected to the potentiostat and the cathode potential was set at -900 mV to evaluate the ability of microorganisms to use the negatively polarized cathode as an electron donor for the production of acetate. At the next stage, the cathode potential was set in the range from -900 to -1,100 mV to test the effect of the set potential on the performance of the BESs. Each test lasted 5 days, and the gas and liquid samples were separately analyzed every day. In parallel, abiotic control tests were also conducted under the same operating conditions, but for the absence of the microbial culture, while control tests without methanogenic inhibitor were also conducted under the same operating conditions, but for the absence of 2-bromoethanesulfonic acid.

Analysis and Calculation

Acetate was analyzed on a C18 column (Welch Materials, Inc.,) using high performance liquid chromatography (Agilent 1260 Infinity LC) at 210 nm (mobile phase of methanol and 10 mM K₂HPO₄ mixed at 7:93 (v/v), a flow rate of 0.8 ml/min, and an oven

temperature of 35°C). Methane and hydrogen gas were detected by Agilent 7890A gas chromatography (GC: 3 m column packed with carbon molecular sieves; Argon carrier gas at 30 ml/min; oven temperature at 30°C; thermal conductivity detector temperature at 80°C). Bacterial morphologies on carbon felt were observed using a scanning electron microscope (SEM; FEI QUANTA200, Holland). Linear sweep voltammetry (LSV) was used to determine the current densities possible in this system with an electrochemical working station (CS120, China) in the potential range from -500 to -1,200 mV at a scan rate of 5 mV/s. Denaturing gradient gel electrophoresis (DGGE) was used to analyze the microbial community of the cathode. DNA extraction, DGGE, cloning, sequencing, and phylogenetic analyses were carried out as previously described [25].

The electron recovery efficiency (ERE) for the formation of product (hydrogen, acetate, and methane) was calculated as follows [28]:

$$\text{ERE} = \frac{n \times f \times F}{\int_0^t I dt} \times 100$$

where *n* is the moles of product (hydrogen, acetate, and methane) harvested; *f* represents the molar conversion factor (2, 8, and 8 eq/mol for hydrogen, acetate, and methane, respectively); *F* is Faraday's constant (96,485 C/mol of electrons); and *I* is the circuit current.

Results and Discussion

Ability of Microorganisms to Produce Acetate

To study the ability of the inoculated microorganisms to produce acetate, the cathode potentials were first set at -900 mV. In the presence of biofilm (Fig. 1A), no hydrogen was detected and no production of methane was observed during the reaction time. The lack of H₂ was probably due to its rapid use by the mixed culture. After 5 days of reaction, acetate accumulated to 1.88 mM. Throughout the test, the cathodic current was maintained at around 6.2 mA. Electrons recovered as acetate amounted to 53.6% (Fig. 1B). In the abiotic control, the current generated was maintained at around 0.04 mA. H₂ production was also low (below 0.05 mM) and almost linearly increased with reaction time. The low hydrogen production in the abiotic tests was probably due to the high overpotential of this reaction at the carbon electrode [28] compared with that using Pt-catalyzed cathodes [2].

Effect of Set Potentials on the Performance of BESs

The impact of set potentials on the performance of BESs, when working electrode potentials were set in the range from -900 to -1,100 mV, is shown in Fig. 2. Hydrogen

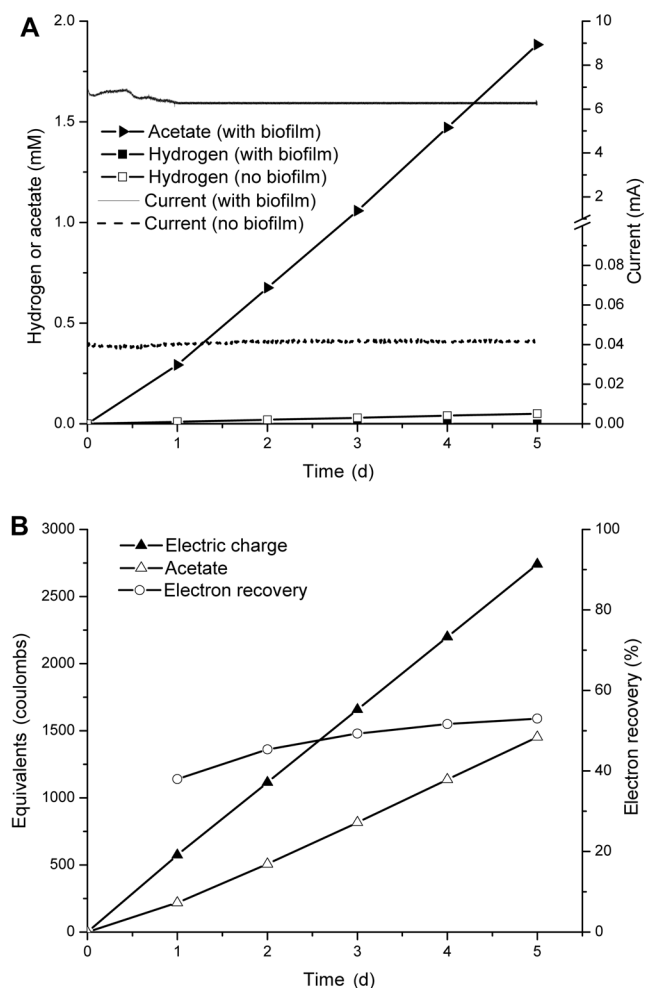


Fig. 1. Bioelectrochemical experiment with addition of methanogenic inhibitor at $V = -900$ mV with enriched mixed culture.

(A) Time course of hydrogen, acetate, and current. (B) Cumulative electric charge, equivalents recovered as acetate, and electron recovery efficiency.

production was low (below 0.24 mM d^{-1}) and no methane was produced either in the biotic test or the abiotic control (Fig. 2A). In the presence of microorganisms, the rate of hydrogen production was similar to that measured in the abiotic tests with the electrode polarized from -900 to $-1,000$ mV; however, at more negative potentials, the observed rate of hydrogen production was significantly lower than in the abiotic tests. This result is similar to those of previous studies [3, 28], due to the rapid rate of interconversion of hydrogen catalyzed by the mixed culture. There was no acetate production in the abiotic tests, whereas in the biotic tests, the rate of acetate production increased as the electrode potential became more negative, from

0.38 mM d^{-1} (-900 mV) to 2.35 mM d^{-1} ($-1,100$ mV). The maximum rate of acetate production was 2.35 mM d^{-1} (0.14 g d^{-1}). Considering the cathode liquid volume is 150 ml , the maximum rate of acetate production was $15.67 \text{ mM l}^{-1} \text{ d}^{-1}$ ($0.94 \text{ g l}^{-1} \text{ d}^{-1}$). This rate of acetate production is 2-20 times higher than previously reported rates for microbial electrosynthesis [18, 19, 30]. The contribution of hydrogen and acetate production reactions to the total electron recovery is presented in Fig. 2B. The electron recovery efficiency of carbon dioxide reduction to acetate increased from 53.6% to 89.5% when the cathode potentials were decreased from -900 to $-1,100$ mV. The equivalents recovered as hydrogen was negligible (below 0.8%) throughout the test. Therefore, the total electron recovery of this process was mostly due to acetate formation. Simultaneous production of acetate and methane from carbon dioxide in BESs was observed by Marshall *et al.* [14] and in our previous study [8]. However, methanogenesis out-competed acetogenesis, and methane has a high global warming potential. With the addition of the methanogenic inhibitor 2-bromoethanesulfonic acid, no methane was detected in batch potentiostatic experiments. Electron recovery in acetate and hydrogen from the 2-bromoethanesulfonic acid-treated community reached 90.3% ($-1,100$ mV). In the control without the methanogenic inhibitor, the maximum rate of acetate production was 0.62 mM d^{-1} (0.04 g d^{-1}). The electron recovery in acetate fluctuated from 0% to 28% when the cathode potential was set from -900 to $-1,100$ mV (Figs. 2C and 2D).

Morphology of Bacteria on the Electrode

Samples were taken after batch potentiostatic experiments and subjected to SEM analysis (Fig. 3). SEMs showed that the cathodes in both sets were densely covered with organisms with different shapes. The cathode to which the methanogenic inhibitor had been added was densely covered with short rod-shaped organisms (Fig. 3A), while the cathode lacking the inhibitor was dominated by long rod-shaped organisms showing a relatively heterogeneous morphology (Fig. 3B). This result is similar to a report in which the operational biocathode was treated with 2-bromoethanesulfonic acid; long rod-shaped microbes became less prevalent while thicker rod-shaped microbes became the dominant morphology on the electrode. Furthermore, the total amount of microorganisms was less on the cathode [14].

LSV of Electrochemical Activity of Microorganisms

As shown in Fig. 4, the current density measured in the presence of the biofilm was significantly higher than that in

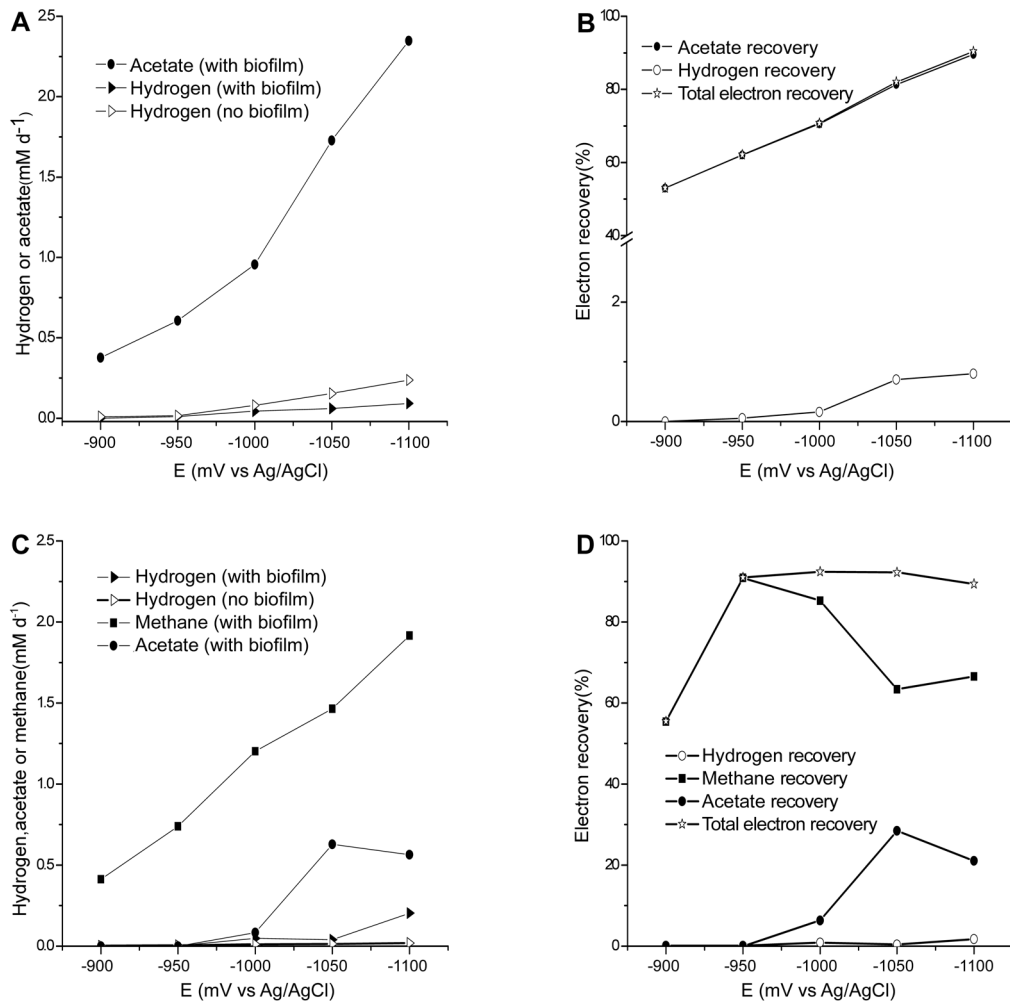


Fig. 2. Bioelectrochemical experiments at different cathode potentials with mixed culture. Formation rates of hydrogen, acetate, and methane with (A) and without (C) methanogenic inhibitor. Electron recovery efficiency with (B) and without (D) inhibitor.

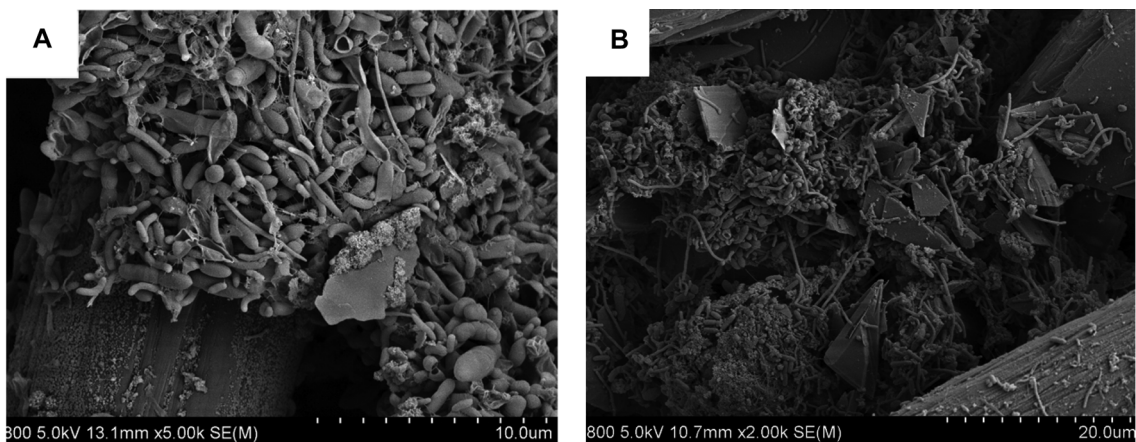


Fig. 3. SEM observations of the bacteria growing on the surface of the biocathodes with (A) and without (B) inhibitor.

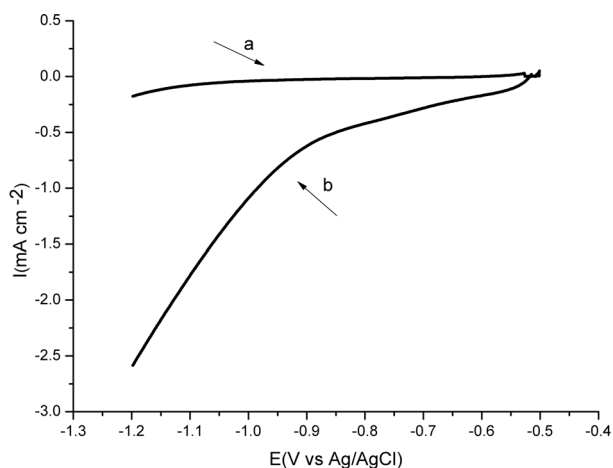


Fig. 4. Linear sweep voltammograms of the cathodes (5.0 mV/s using CO₂-saturated medium).

(a) Without biofilm. (b) With biofilm.

the abiotic control when the electrode potential was more negative than -750 mV. This result suggests that the microorganisms were more active when the electrode potential was more negative and that they catalyzed the formation of product by directly accepting electrons from the surface of the electrode and/or by utilizing the hydrogen gas generated abiotically [28].

Molecular Characterization of the Biocathode in BESs

To determine the electro-active microbial community response for carbon dioxide reduction, DGGE was conducted (Fig. 5). BLASTn searches showed that the cathode of BESs in the presence of methanogenic inhibitor was dominated by relatives of *Advenella mimigardefordensis* (band 1), *Acetobacterium woodii* (band 2), *Arcobacter cibarius* (band 3), and *Wolinella succinogenes* (band 4). *A. woodii* and *W. succinogenes* were both enriched in the cathodes of BESs in the presence and absence of the inhibitor. Pure cultures of *W. succinogenes* have shown perchlorate-degrading activity in the presence of added hydrogen [29]. Members of the genus *Wolinella* were also present in BESs for the simultaneous production of methane and acetate [14]. *A. woodii* is a Na⁺-dependent acetogen, lacking cytochromes but having membrane-bound corrinoids [16] and a carbon-conserving mechanism by means of a Na⁺-dependent ATPase [4]. It has been suggested that the acetogen *A. woodii* is unable to consume current in BESs [18]. It is reasonable to conclude that *A. woodii* acted as the sole microorganism to catalyze the formation of acetate by utilizing the hydrogen gas generated abiotically in this study. The working principles

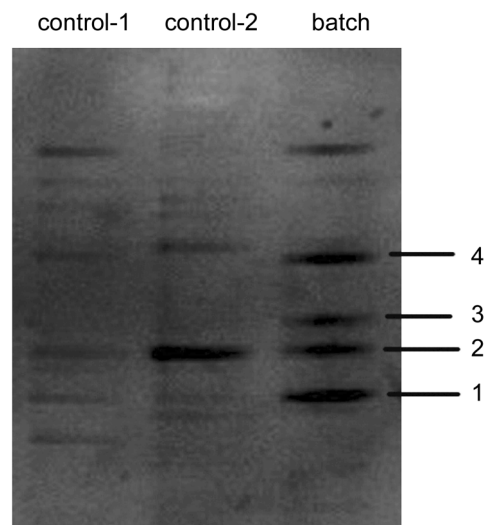


Fig. 5. DGGE profiles of 16 S rRNA fragments amplified from the biocathode and inoculum.

Control-1: inoculum; control-2: without methanogenic inhibitor; batch test: with inhibitor.

and the possible pathways for the entire process of the bioelectrochemical system for the production of acetate are presented in Fig. 6. At more negative potentials from -900 to -1,100 mV, the amount of hydrogen gas generated became more sufficient for the conversion to carbon dioxide by microorganisms. The hydrogen in the recycled gas of the cathode chamber was below 1% and no hydrogen inhibition occurred [7]. Thus, the electron recovery efficiency of carbon dioxide reduction to acetate increased.

When the methanogenic inhibitor was added to the BESs initially, hydrogen-utilizing microorganisms became attached to the cathode through the process. The cathode

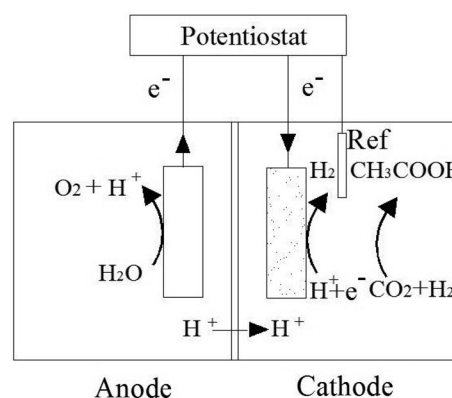


Fig. 6. Proposed scheme for acetate production from carbon dioxide in the presence of methanogenic inhibitor.

was densely covered with microorganisms but the populations were stable and monotonous. Although the cathode potential is a critical factor in the performance of the BESs, other factors, including the presence of toxic chemicals (*i.e.*, sulfide) [27], pH [10, 20] and the electrode material [30], may need to be examined in future studies to better understand and optimize the performance of BESs. The overall advantages of BESs for wastewater treatment and biofuel production from carbon dioxide could make them an important method for bioenergy production in the future.

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