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Carbon dioxide reduction by mixed and pure cultures in microbial electrosynthesis using an assembly of graphite felt and stainless steel as a cathode

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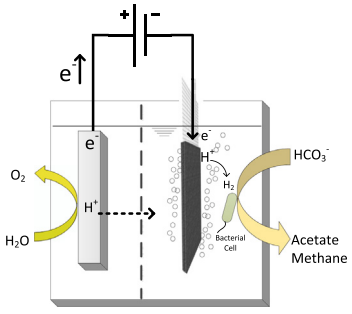
HIGHLIGHTS

- Stainless steel and graphite felt assembly as a CO_2 reducing biocathode material.
- Higher acetate production rates from *C. ljungdahlii* with hydrogen evolution.
- CH_4 production predominated from CO_2 reduction by non-enriched mixed culture.
- H_2 evolution appears to stimulate planktonic bacteria rather than cathodic-biofilm.

GRAPHICAL ABSTRACT



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ABSTRACT

Carbon dioxide (CO_2) reduction to multi-carbon compounds at the cathode using chemolithoautotrophs is an emerging application of microbial electrosynthesis (MES). In this study, CO_2 reduction in MES was investigated at hydrogen evolving potentials, separately by a mixed culture and *Clostridium ljungdahlii*, using a graphite felt and stainless steel assembly as cathode. The mixed culture reactor produced acetate at the maximum rate of 1.3 mM d^{-1}, along with methane and hydrogen at -1.1 $V_{/Ag/AgCl}$. Over 160 days of run-time in four fed-batches, 26% of bicarbonate was converted to acetate between day 28 and 41, whereas in the late batches, methane production prevailed. Out of 45 days of run-time in the *C. ljungdahlii* reactor, 2.4 mM d^{-1} acetate production was achieved at -0.9 $V_{/Ag/AgCl}$ in Batch 1. Simultaneous product degradation occurred when the mixed culture was not selectively enriched. Hydrogen evolution is potentially the rapid way of transferring electrons to the biocatalysts for higher bioproduction rates.

1. Introduction

Bioelectrochemical systems (BESs) offer unique possibilities for the clean and efficient production of high-value chemicals and fuels from low-value wastes or even carbon dioxide (CO_2) using microorganisms as biocatalysts (Nevin et al., 2010; Rabaey and Rozendal, 2010), such systems are referred to as microbial electrosynthesis (MES). CO_2 can be metabolically reduced to multi-carbon organic compounds by microbes using electrons or reducing equivalents derived from the cathode in MES. An oxidation reaction at the anode produces protons and electrons for the cathodic reduction and an electric power source drives the electrons from anode to cathode through an external circuit.

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Several lithoautotrophs have been reported for the metabolic reduction of CO_2 and carbon monoxide (CO) to acetate and other multi-carbon compounds with hydrogen (H_2) as energy source (Amils, 2011). The anaerobic conversion of CO_2 and H_2 to acetate by acetogenic bacteria has been described to follow the reductive acetyl-CoA pathway (Ljungdahl, 1986). In the case of MES, reduction at the biocathode occurs with the application of electric energy without the external supply of reductants such as hydrogen. The microbes involved in the biocathode can receive the electrons either directly from the cathode or indirectly via mediators or via H_2 produced by water electrolysis (Lovley, 2011; Rabaey and Rozendal, 2010). Nevin et al. (2010) described the first proof of principle of CO_2 reduction in microbial electrosynthesis using the acetogen *Sporomusa ovata*, which can use electrons directly from solid graphite electrodes for the reduction of CO_2 to produce acetate and small amounts of 2-oxobutyrate at -0.6 $V_{Ag/AgCl}$ cathode potential. In a succeeding study, a number of pure acetogenic cultures namely, *Sporomusa*, *Clostridia* and *Moorella* sps. demonstrated microbial electrosynthesis by reducing CO_2 directly using electrons from the electrode at -0.6 $V_{Ag/AgCl}$ cathode potential (Nevin et al., 2011). Mixed microbial consortia from different sources have also been used in MES for CO_2 reduction to acetate (Jiang et al., 2013; Marshall et al., 2012; Su et al., 2013; Zaybak et al., 2013). In addition to the acetate, methane was also produced as a by-product when mixed culture inoculum was used without pre-enrichment (Jiang et al., 2013; Marshall et al., 2012).

Thermodynamically, the threshold potential for hydrogen evolution at a cathode is -0.6 $V_{Ag/AgCl}$ at pH 7 under biological conditions but the threshold potential shifts further to more negative magnitudes due to the electrode overpotentials. When the bacteria reduce CO_2 at less negative cathode potential than that required for hydrogen evolution, direct electron uptake from electrode could potentially occur. Direct electron transfer at less negative cathode potential is interesting and ideally energy-efficient in bioelectrocatalysis (Lovley, 2011) but the volumetric production rates and yields reported in such studies were fairly low. In particularly, Nevin et al. (2010) reported only ca. 0.17 mM d^{-1} acetate production with *S. ovata* at -0.6 $V_{Ag/AgCl}$ with ~1 L of catholyte spent under the continuous operation and Zaybak et al. (2013) reported only 0.05 mM d^{-1} acetate production with enriched mixed culture at -0.6 $V_{Ag/AgCl}$. Nickel nanoparticles coating on carbon based cathode improved the surface-based production rates in *S. ovata* MES systems (Nie et al., 2013; Zhang et al., 2013). Projected surface area based acetate production was increased 2.3 times over untreated graphite cathode using Ni nanowire coating on graphite stick at -0.6 $V_{Ag/AgCl}$ (Nie et al., 2013). However, the volumetric production rate remained only 1.13 mM d^{-1} even with the surface modifications.

For the upscaling of MES, an increase in the electrode/electrolyte ratio and a better acclimated biocathode can consequently increase the production rates. Indeed, application of a slightly more negative cathode potential for bioelectrochemical CO_2 reduction seem to be favorable for higher conversion rates and titers (Marshall et al., 2013). Improvements in acetate production from CO_2 reduction were shown using mixed microbial communities at more negative cathode potentials along with H_2 and methane (CH_4) production (Jiang et al., 2013; Marshall et al., 2012). At more negative potentials, proton reduction is favored producing H_2 which mediates the electron transfer to CO_2. But, the evolved H_2 (in the gaseous phase) might not be completely available for biological CO_2 reduction and ultimately, escapes from the reactor, lowering the current and energy-input efficiencies of the process. An optimization among the production rates, H_2 availability in the catholyte and efficiencies could be achieved by using a highly adsorbing cathode materials with lower H_2 evolution overpotential. Using granular carbon bed cathode, Marshall et al. (2013) achieved highest acetate production rate of 17.25 mM d^{-1} at -0.79 $V_{Ag/AgCl}$ along with H_2 production from methanogenesis suppressed autotrophic microbiomes when acclimated for long time in MES mode. Recently, Jourdin et al. (2014) reported highest surface area based acetate production rate (1.3 ± 0.2 mM cm^{-2} d^{-1}) with nanoweb reticulated vitreous carbon cathode at -1.05 $V_{Ag/AgCl}$ but still the volumetric production rate remained only ~0.5 mM d^{-1}.

Generally, anaerobic biofilm development at negatively polarized biocathode, feeding only bicarbonate (HCO_3^-) is difficult without addition of H_2 and/or organic compounds (Gregory et al., 2004). Autotrophic growth of acetogens is occurring at the theoretical thermodynamic microbial metabolic limits (Schuchmann and Müller, 2014). Thus, electrochemical H_2 production at more negative cathode potential in an MES appears to hold the key for the stimulation of autotrophic metabolism of hydrogenotrophic acetogens in the biocathode (Rabaey and Rozendal, 2010). Moreover, H_2 adsorbed on the electrode surface or dissolved in the electrolyte serves as electron shuttle in microbial CO_2 reduction. Most recently, the electrochemically produced H_2 driven MES has also been reported for producing high-value products such as butyrate from CO_2 reduction (Ganigué et al., 2015).

The main goal of this study is to investigate the application of lower H_2 evolution overpotential material like stainless steel in an assembly with graphite felt as cathodes in CO_2 reduction in MES using mixed and pure microbial cultures. The study also provides a comparative scenario of MES using mixed and pure bacterial cultures with respect to the competing biological processes involved and their response to the electrochemical H_2 evolution. The MES performances in long-term batch operations are evaluated based on current efficiencies, production rates and current densities.

2. Materials & Methods

2.1. Bacterial cultivation

For the pure culture experiments, a homoacetogenic bacterial strain *Clostridium ljungdahlii* DSM 13528 was purchased from the German Collection of Microorganisms and Cell Cultures DSMZ (Germany). *C. ljungdahlii* cells were cultivated anaerobically in serum bottles at 37 °C in DSMZ 879 medium for at least two weeks. Bacterial growth was attained with optical density at 600 nm OD_{600} reached 0.3–0.4 and the culture strain was verified by microscopic tests. The bacterial strain was maintained viable by sub-culturing in every 2–3 months until the MES reactors were inoculated. The sub-culturing was done up to four batches.

The inoculum for mixed culture MES was obtained from an anaerobic culture of carboxydotrophic actinomycete *Streptomyces thermospinisporus* DSM 41779 in serum bottles in Nutrient Buffer Acetate–Fumarate (NBAF) medium (Coppi et al., 2001), which was mixed with the wastewater sludge available in the laboratory during the anaerobic sub-culturing. The mixed culture community was undefined but when cultivated on mineral medium (DSMZ 879 medium without fructose) with H_2:CO_2 (80:20 v/v) gas in headspace at 1.5 bar, it grew to an OD_{600} of 0.2 indicating the presence of CO_2 fixing strains. After two anaerobic sub-culturing in mineral media with H_2:CO_2 (80:20) gas in headspace, the mixed culture was inoculated into the catholyte of MES reactor.

2.2. Bioelectrochemical reactor setups

2.2.1. Setup for mixed culture

For the experiments with mixed cultures, a double chambered bioelectrochemical cell was assembled using two circular