Single-chamber microbial electrochemical cell for CH₄ production from CO₂ utilizing a microbial consortium

Hannah Giang¹ | Ji Zhang² | Zeying Zhu² | Ian I. Suni¹,³,⁴ | Yanna Liang²,⁴

¹ Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, IL 62901, USA
² Department of Civil and Environmental Engineering, Southern Illinois University, Carbondale, IL 62901, USA
³ Department of Mechanical Engineering and Energy Processes, Southern Illinois University, Carbondale, IL 62901, USA
⁴ Materials Technology Center, Southern Illinois University, Carbondale, IL 62901, USA

Correspondence
Ian I. Suni, Materials Technology Center, Southern Illinois University, Carbondale, IL 62901, USA.
Email: isuni@siu.edu

Summary
We report the first single-chamber microbial electrochemical cell for conversion of CO₂ to CH₄, with an average CH₄ production rate of 0.47 ± 0.05 mL day⁻¹ cm⁻² at an applied potential of 600 mV, utilizing a methanogenic microbial community collected from the formation water in the San Juan coal basin (Colorado, USA). CH₄ production was only observed at the graphite rod cathode after an electrochemical pre-treatment that facilitates biofilm formation. The carbon contained within the CH₄ arose predominantly from the CO₂ source, as verified by experiments during which the CO₂ source was repeatedly turned off and on. Modest quantities of acetic acid and ethanol were also produced. DNA extraction and sequencing from the microbial community showed that from the Archaea kingdom, only 2 species survived prolonged exposure to CO₂ and CH₄ production, *methanobacterium* sp. (81.4%), and *methanoculleus* sp. (18.6%), while in the bacterial kingdom, *anaerobaculum thermoterrnum* (67.1%) was the predominant surviving species.

KEYWORDS
bioelectrochemistry, electrode pretreatment, methane production, microbial consortium, microbial electrochemical cell

1 | INTRODUCTION

CO₂ emission reduction is paramount due to widespread concern regarding their contribution to climate change.¹,² Although CO₂ contains strong covalent bonds and is highly stable under most conditions, conversion of CO₂ to hydrocarbons has been reported by chemical, photochemical, electrochemical, and biological methods.³,⁴ Economic benefits accrue from conversion of CO₂ to liquid phase chemicals such as formic acid, acetic acid, urea, ethanol, and methanol, or to gases such as methane, carbon monoxide, and acetylene. However, many of these methods employ hydrogen as a reducing agent and might therefore not be economical. In addition, because most hydrogen is derived from fossil fuels, this mitigates the otherwise positive impact on reducing climate change.⁵

Microbial electrochemical cells (MECs) have been widely investigated for conversion of biological waste products into hydrogen,⁶,⁷ where reduction reactions at the cathode involve combined electrochemical and microbial processes. However, MEC technology has been expanded to other applications, including reduction of CO₂ to chemicals such as formic acid and acetate,⁸-¹³ and quite recently reduction of CO₂ to CH₄.¹⁴-¹⁷ A significant advantage of MECs relative to other technologies is that an external potential and cellular metabolism may be substituted for hydrogen as the reducing agent.

Most MECs are designed as 2-chamber systems, with the anode and cathode chambers separated by either an anion or cation exchange membrane, depending on the desired product.¹⁸ The membrane allows separate optimization of the cathode-catholyte and anode-anolyte, because the
cathode and anode reactions will respond differently to changes in pH, ionic strength, convective mixing, and the presence of nutrients and other chemicals. In addition, the membrane prevents unwanted species crossover, such as oxygen produced at an aerobic anode diffusing into an anaerobic cathode chamber, or the desired product from the cathode diffusing into the anode chamber. However, separation of the cathode and anode chamber with a membrane introduces several problems, such as possible ohmic loss in the membrane, and increased cost and complexity. In addition, substantial pH gradients may develop across membranes, resulting in a potential loss of 0.06 V per pH unit. The objective of the current study is to develop and report a single-chamber MEC for conversion of CO₂ to CH₄ with high yield and high efficiency.

2 | MATERIALS AND METHODS

2.1 The microbial community and medium

A methanogenic microbial community collected from the formation water in the San Juan coal basin (Colorado, USA) is used in this study. This community, originally obtained from coal from the San Juan basin, was maintained anaerobically at 41°C (the temperature at the basin) in a methanogen standard (MS) medium that includes trace minerals. To start the enrichment process, the MS medium and tryptic soy broth (TSB, 30 g/L) medium were used as described below. In addition, a defined medium (DM) was used later that contains the ingredients in the MS medium minus yeast extract and peptone, but with addition of 0.1 ml/L of vitamin solution. Thus, the DM contains no carbon sources.

2.2 Microbial electrochemical cell and its operation

Borosilicate glass bottles (100 mL) served as the single-chamber MEC, as shown in Figure 1, where graphite rods (99.9995% C) were used as both the cathode and anode. The cathode (4 cm in length × 0.305 cm in diameter) was longer than the anode (2 cm long × 0.305 cm in diameter) so that the cathode only could be embedded within the graphite granules on the reactor bottom.

Prior to use, graphite rod cathodes were cleaned in an ultrasonic bath with Alconox, concentrated HNO₃, acetone, and ultrapure water for 15 minutes each to remove contamination. For some experiments, this was followed by electrochemical activation with 300 potential cycles at 50 mV/s between +1.5 V and −0.4 V (vs Ag/AgCl) in an electrolyte containing 1.0 M HNO₃ and 0.10 M NaF. This procedure both increases the surface roughness and introduces oxygen functionality onto the graphite cathode. The graphite anodes were then rinsed with ultrapure water and autoclaved at 121°C for 1 hour. Graphite granules were submerged for 24 hours in 37% HCl, submerged for 24 hours (3 successive times) in 1.0 M NaOH, rinsed with ultrapure water, and then dried thoroughly at 100°C. However, the graphite granules were not electrochemically activated using the procedure above that was applied to the graphite rod cathode.

Each sealed MEC contained 10-g pretreated graphite granules, 45 mL of medium, and 5 mL of the microbial community. Titanium wires were inserted through rubber stoppers to make electrical contacts to both the cathode and anode. The MEC reactors were incubated in a water bath at a constant temperature of 41°C. A potential of 600 mV was applied with a power supply to increase the
CO₂ reduction rate. Eight MEC reactors were operated in parallel to multiplex these experiments, so power supplies were more convenient to use than potentiostats. At the end of some experiments, the cathode was imaged by scanning electron microscopy (SEM) in an FEI Corp. Quanta FEG 450.

The headspace gas was sampled periodically by inserting a stainless steel needle into the reactor headspace to release overpressure arising from microbial activity and maintain a constant pressure of 1.0 atm. The other end of the needle was connected to a 50-mL gas tight syringe, and the gas volume was recorded. In addition, the molar composition of CH₄ and CO₂ in the reactor headspace was analyzed with a Shimadzu 17A gas chromatograph equipped with a 60 m × 0.53 mm RT-Msieve 5 porous layer molecular sieve using Ar as the carrier gas. Products in the culture medium (ie, formic acid, acetic acid) were analyzed by reverse phase Shimadzu HPLC by establishing a calibration curve for each compound of interest.

2.3 | DNA extraction and sequencing analysis

DNA from the electrolyte was extracted using Powerwater DNA extraction kit, and high quality DNA samples were then sequenced. To understand the diversity of the microbial population, the 16S rRNA gene V4 variable region PCR primers 515/806 were used. Single-step PCR using the HotStarTaq Plus Master Mix Kit was performed at 94°C for 3 minutes, followed by 28 cycles lasting 30 seconds each; 53°C for 40 seconds; 72°C for 1 minute, and 72°C for 5 minutes. Sequencing was conducted at Molecular Research on an Ion Torrent PGM following the manufacturer’s guidelines, and they also processed sequence data (15–20 000 reads/assay). Operational taxonomic units were defined as clusters at 3% divergence (97% similarity).

3 | RESULTS AND DISCUSSION

3.1 | Methane yield from microbial consortium

The enrichment process began with TSB medium, followed by medium replacement with TSB, then replacement with MS, DM, and DM sequentially every 10 days during the experiments in order to maintain sufficient nutrition for cell growth. The TSB medium was used initially to enrich the methanogenic bacteria population. As will be discussed later, CH₄ production was obtained in the MEC only when the graphite cathode rod was electrochemically pretreated by the potential sweep method described in the Experimental section. Figure 2 shows the gas chromatography results for headspace sampling at day 38, illustrating that predominantly methane is produced in the gas phase. Gas content on other days showed similar results but with different methane concentrations.

Figure 3 shows the methane yield as a function of time during one 64-day trial. Surprisingly, the methane production rate was consistent in different culture mediums during the entire trial. These data were fit by linear regression to give an average methane production rate of 0.47 ± 0.03 mL day⁻¹ cm⁻², which ranges from 4 times to 360 times higher than that reported in 2-chamber MECs. Several research groups have reported related MEC systems for CH₄ production from other carbon
sources such as bicarbonate, acetate, and glucose. There are several explanations for the high methane production rate reported here. First, the single-chamber MEC avoids the potential drop across the membrane separating the catholyte and anolyte in a 2-chamber MEC, so chemical-electrochemical potential energy can be utilized more efficiently.

In addition, omission of this membrane provides methanogenesis bacteria with more direct access to the protons (H⁺) produced along with O₂ during the anode reaction (2) below. Because methanogens utilize H⁺ to convert CO₂ to CH₄, better access to anodically produced H⁺ significantly increases the methane production rate within a single-chamber MEC. The likely cathodic and anodic reactions are given in Equations 1 and 2:

$$CO₂ + 8H^+ + 8e^- \rightarrow CH₄ + 2H₂O \quad (1)$$

$$2H₂O \rightarrow 4H^+ + O₂ + 4e^- \quad (2)$$

Of course, simultaneous 8-electron transfer reactions are unlikely, and reaction (1) has been reported to occur within methanogens through a complex multi-step metabolic pathway.

In Figure 2, significant H₂ production at the cathode is not observed in the single-chamber MEC, even though H₂ is easily detected by gas chromatography. Hydrogen evolution occurs at a lower overpotential than CH₄ production, and significant production of H₂ has been reported in 2-chamber MEC experiments, resulting in low CH₄ production (0.1–0.2 mL/week). Methane production at the cathode can occur either through direct reduction where microorganisms directly utilize electrons from the power source along with protons from the catholyte for CO₂ reduction, or by indirect reduction in which H₂ is initially generated and subsequently converted by reaction with CO₂ to form CH₄. The lack of observable H₂ formation at the cathode of the single-chamber MEC suggests that H⁺ is rapidly harvested by microorganisms for CH₄ production. Moreover,
the lack of conversion to H2 improves current efficiency for methane production.

3.2 | Enriched microbial consortium utilizes CO2 as carbon source

The ability of the microbial consortium to produce methane at a high rate is surprising in a DM that contains no carbon sources. At first glance, CO2 appears to be the only carbon source available for continuous methane production. To test this hypothesis, culture cycles were examined where the culture medium was refreshed and fresh CO2 was purged. The amount of CO2 and CH4 in the head space of the reactors was measured during each culturing cycle, as shown in Figure 4. The concentrations of CH4 and CO2 are clearly anti-correlated, beginning with the second culturing cycle. During the first culturing cycle, the overall content of CO2 and CH4 decreases substantially, possibly because species other than methanogens in the original microbial community convert CO2 into other products. However, these species appear to be gradually eliminated during these experiments, and the adapted microbial organisms are mainly methanogenic bacteria, because methane becomes the primary product after the initial enrichment phase.

3.3 | Importance of cathode pre-treatment

Effective biofilm formation is likely a pre-requisite for microbial utilization of the applied potential (600 mV) for CO2 reduction at the cathode in the single-chamber MEC. The importance of electrode pre-treatment on biofilm formation was tested by comparing the methane production rate, with and without graphite rod cathode pretreatment by 300 potential sweeps at 50 mV/s between +1.5 V and −0.4 V. (vs Ag/AgCl) in an electrolyte containing 1.0 M HNO3 and 0.1 M NaF. Figure 5A illustrates the methane production yield at the graphite cathode without pretreatment, which averages ~0.007 ± 0.002 mL day⁻¹ cm⁻². The difference between the methane production rates in Figure 5A,B can be understood from the greatly improved biofilm formation on the electrochemically pre-treated graphite cathode relative to the un-treated cathode. Figure 6A,B shows SEM images of the pre-treated and untreated cathodes at the end of MEC operation. Clearly, biofilm formation is much more complete in Figure 6B for the electrochemically pre-treated graphite cathode, which allows microbes more direct access to the electrons from the external power source. Possible biofilm formation on the graphite granules at the bottom of the cathode was not investigated, but the graphite granules are inactive towards CH4 production. They are present (without activation) for all experiments, and significant CH4 production is only observed when the graphite rod cathode is electrochemically activated.

3.4 | Enriching with MS standard medium

Tryptic soy broth medium is a rich medium for rapid cell growth but is relatively expensive. MS medium contains less nutrition relative to the TSB medium but is relatively inexpensive. Therefore, enrichment experiments starting directly with MS medium followed by DM were also performed. The results show a much lower methane production rate of 0.20 ± 0.02 mL day⁻¹ cm⁻² (Figure 5B) compared with 0.47 ± 0.03 mL day⁻¹ cm⁻² when starting with TSB medium. Thus, the initially rapid growth of bacteria is essential for enrichment of methanogenous bacteria, thus providing rapid methane production.

FIGURE 6 SEM images of graphite rod cathodes following single-chamber MEC reactor operation with (A) and without (B) electrochemical cathode pretreatment [Colour figure can be viewed at wileyonlinelibrary.com]
3.5 | Liquid phase products and methane selectivity

Clearly, the overall cathodic reaction given in Equation 1 above occurs in many successive steps due to the large number of electrons and protons transferred, so side reactions might be expected. In addition, the microbial community contains different species with different metabolisms. Organic compounds that might be expected to form include formic acid, acetic acid, urea, ethanol, and methanol. Therefore, 2 mL of the medium was removed and analyzed by HPLC at the end of a 64-day trial that started with the TSB medium. The results show that both acetic acid and ethanol can be detected at concentrations of 1.5 and 0.007 g/L, respectively. Comparing the final concentrations of acetic acid and ethanol to the methane production rate, the methane selectivity can be estimated as ~62%. This is roughly compatible with our estimate of the Faradaic current efficiency of ~70%. The Faradaic current efficiency is estimated from $\eta_{CH_4} (%) = \frac{n_{CH_4}}{it} \times 100$, where $n_{CH_4}$ is the number of moles of methane produced, and $it$ is the total charge from power supply during the experiment. The selectivity of methane was estimated from $S_{CH_4} = \frac{n_{CH_4}}{n_C} \times 100 = \frac{n_{CH_4}}{(n_{CH_4} + n_{CH_3COOH} + n_{CHCHO})} \times 100$.

3.6 | Composition of the adapted methanogenic microbial communities

Based on 16S next generation DNA sequencing, the microbial community prior to MEC operation consisted of 68% bacteria and 32% archaea. The former is distributed among 294 species within 61 orders and the latter among 13 species within 5 orders. The 3 dominant bacterial orders are Thermoanaerobacterales (16.1%), Synergistales (13.9%), and Bacillales (13.8%). Within the Archaea kingdom, the order of Methanobacteriales, Methanosarcinales, Methanomicrobiales, Methanocellales, and Thermoproteales are 96.1%, 2.5%, 1.0%, 0.2%, and 0.2%, respectively. Culture media with nitration, including organic compounds such as acetate, are typically employed to grow microorganisms in an MEC reactor. Here, the methane production rate remains quite high in the DM, even though this contains no organic carbon or nitrogen sources. Thus, it is important to identify which bacterial species from these microbial communities thrive in the DM.

From the Archaea kingdom, only 2 species survived prolonged exposure to DM, *methanobacterium sp.* (81.4%), and *methanoculleus sp.* (18.6%), as shown in Figure 7A. This is consistent with production of mainly CH$_4$, as described earlier, because these *methanobacterium* sp. have previously been reported to convert CO$_2$ to CH$_4$. On the other hand, the DNA sequencing results shown in Figure 7B show that bacteria in the microbial community in DM are dominated by *anaerobaculum thermoterrum* (67.1%). Other bacterial species such as *coprothermobacter*
proteolyticus (7.6%), coprothermobacter spp. (7.0%), clostridium sp. (8.2%), and thermacetogenium spp. (3.3%) are also observed. Anaerobaculum thermoterrum are active bacteria well known to produce acetic acid in the anaerobic environment as in this experiment setup, which explains the acetic acid production observed in the DM.

4 | CONCLUSIONS

A single-chamber MEC was successfully demonstrated for the enrichment of a microbial consortium that converts CO$_2$ to CH$_4$ with an average production rate of 0.47 ± 0.05 mL day$^{-1}$ cm$^{-2}$ at an applied potential of 600 mV with high current efficiency (~70%). Surprisingly, the microbial community adapted well to a DM that lacks any carbon sources and is able to actively convert CO$_2$ to CH$_4$. Two important factors are identified for high rate methane production, electrochemical activation of the graphite rod cathode to enable biofilm formation and a 2-step enrichment process, where a rich medium such as TSB facilitates an initial rapid increase in cell population, but eventual replacement with a DM facilitates cell adaptation to their environment. By-products such as acetic acid and ethanol are also obtained, and the methane selectivity is estimated as ~62%. Active species in the enriched microbial consortium were identified by DNA sequencing analysis, and methanobacterium sp. (81.4%) were the dominant archae species, so the high production efficiency and selectivity for methane in these experiments can be explained by the high population of methanogenic bacteria in the adapted microbial community.

CONFLICTS OF INTEREST

The authors declare that they do not have any conflicts of interest.

ORCID

Ian I. Suni http://orcid.org/0000-0002-6889-8158

REFERENCES


