Bioelectrochemical removal of carbon dioxide (CO₂): An innovative method for biogas upgrading

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HIGHLIG T H S

- Bioelectrochemical removal of CO₂ for biogas upgrading showed great potential.
- The in-situ biogas upgrading system was more effective than the ex-situ one.
- Species from the genus Methanobacterium were dominant on all the biocathodes.
- Alkali production and CO₂ absorption might also contribute to biogas upgrading.

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ABSTRACT

Innovative methods for biogas upgrading based on biological/in-situ concepts have started to arouse considerable interest. Bioelectrochemical removal of CO₂ for biogas upgrading was proposed here and demonstrated in both batch and continuous experiments. The in-situ biogas upgrading system seemed to perform better than the ex-situ one, but CO₂ content was kept below 10% in both systems. The in-situ system's performance was further enhanced under continuous operation. Hydrogenotrophic methanogenesis and alkali production with CO₂ absorption could be major contributors to biogas upgrading. Molecular studies showed that all the biocathodes associated with biogas upgrading were dominated by sequences most similar to the same hydrogenotrophic methanogen species, Methanobacterium petrolearium (97–99% sequence identity). Conclusively, bioelectrochemical removal of CO₂ showed great potential for biogas upgrading.

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1. Introduction

Biogas produced from wastes, residues and energy crops is a reliable, flexible and renewable energy source (Holm-Nielsen et al., 2009). It can replace fossil fuels needed for heat and power generation (Weiland, 2010); and, if properly upgraded, it can also be used as vehicle fuel and as a natural gas substitute (bio-natural gas) (Holm-Nielsen et al., 2009; Appels et al., 2011).

Raw biogas is composed of 60–70% CH₄ and 30–40% CO₂ but for biogas to work as an effective fuel source, the ratio of methane to CO₂ needs to be increased. Therefore, such physiochemical and ex-situ treatment methods as pressure swing adsorption (PSA), absorption and membrane separation techniques (Rycroebosch et al., 2011) have been developed to remove CO₂ from raw biogas. While these techniques are effective at CO₂ removal, they not only require complicated operating systems but produce unwanted end products that need further treatment. Several in-situ strategies have been developed, which take advantage of the fact that CO₂ is much more soluble than CH₄ in the liquid phase, especially under high-pressure conditions (Wang et al., 2003). The most cost-effective and commonly used approach involves the addition of a side-stream CO₂ desorption unit alongside the anaerobic digester, through which a liquid stream from the digester is circulated (Hayes et al., 1990; Nordberg et al., 2012). By operating the anaerobic digester at elevated auto-generated pressure, CO₂ can be selectively enriched in the liquid phase while keeping CH₄ in the gas phase (Lindeboom et al., 2011). However, they pose certain technical challenges such as CH₄ loss and disposal of the retained CO₂ (Lindeboom et al., 2011; Nordberg et al., 2012).

It has been proposed that biological processes can be used to overcome some of these technical challenges (Luo and Angelidaki, 2013; Wang et al., 2013). For example, a
chemo-autotrophic methanogen (Methanobacterium thermoautotrophicum) has been shown to upgrade biogas biologically by removing CO₂ and H₂S, and almost doubling the original CH₄ mass (Strevett et al., 1995). Similarly, enriched hydrogenotrophic methanogens from mixed anaerobic cultures were able to convert almost all (~91%) of the CO₂ in raw biogas to CH₄ (Luo and Angelidaki, 2013).

Another approach that shows promise for biogas upgrading involves the use of bioelectrochemical systems that allow microorganisms to convert CO₂ into CH₄ when electricity and water are provided as the only energy and electron sources (Lovley and Nevin, 2013; Van Eerten-Jansen et al., 2012). CO₂ is biologically removed from bioelectrochemical systems in one of two ways. In a process called “electromethanogenesis,” methanogens can directly accept electrons from electrodes for CO₂ reduction (CO₂ + 8H⁺ + 8e⁻ → CH₄ + 2H₂O) (Cheng et al., 2009; Villano et al., 2010). While electromethanogenesis has not been well confirmed or characterized, a recent study has shown that Methanoseta species, which are abundant in anaerobic digesters, are capable of direct interspecies electron transfer (DIET) (Rotaru et al., 2014). Another group of microorganisms, hydrogenotrophic methanogens, can remove CO₂ from the system by using H₂, produced electrochemically or bioelectrochemically, as an electron donor for methane production (CO₂ + 4H₂ → CH₄ + 2H₂O) (Van Eerten-Jansen et al., 2012). DIET is expected to be more metabolically favorable and energy-efficient than interspecies electron transfer (via hydrogen) (Rotaru et al., 2014; Van Eerten-Jansen et al., 2012). Nevertheless, both electro- and hydrogenotrophic methanogenesis contribute to the removal of CO₂.

This study focuses on the bioelectrochemical removal of CO₂ as an innovative method for biogas upgrading. Its feasibility in both in-situ and ex-situ biogas upgrading systems was investigated in batch and continuous modes. Furthermore, microbial biocathodes from both the batch and continuous experiments were characterized and compared in terms of biomass attached and archaeal community composition.

2. Methods

2.1. Batch experiments

The experiments were performed in the same H-cell devices (Fig. 1a) as previously described (Bond and Lovley, 2003). As shown in Fig. 1a, for the ex-situ biogas upgrading system, a 58 mL anaerobic serum bottle served as the digester. Biogas produced from this digester was transferred into the H-cell’s cathode chamber for upgrading. In contrast, simultaneous biogas production and upgrading was achieved in the cathode chamber (digester) of the in-situ system.

The experiments were performed in the same H-cell devices (Fig. 1a) as previously described (Bond and Lovley, 2003). As shown in Fig. 1a, for the ex-situ biogas upgrading system, a 58 mL anaerobic serum bottle served as the digester. Biogas produced from this digester was transferred into the H-cell’s cathode chamber for upgrading. In contrast, simultaneous biogas production and upgrading was achieved in the cathode chamber (digester) of the in-situ system.

![Fig. 1a. Experimental systems for biogas upgrading in batch mode. The working (cathode), counter (anode) and reference electrodes were connected to a potentiostat and the cathode potential was poised at ~700 mV (versus SHE). The working volume of the cathode chambers was 0.2 L.](image)

2.2. Continuous experiments

The continuously fed in-situ biogas upgrading was performed in an integrated anaerobic reactor (Fig. 1b). As shown in Fig. 1b, one chamber from the 1 L H-cell was employed as the cathode chamber (digester) with a working volume of 0.8 L. The cylinder (200-mm height, 30-mm inside diameter) inside the digester was sealed with epoxy resin, and it served as the anode chamber. The electrodes used here were the same as in batch experiments.

The digester was inoculated with 2.0 g (based on TS) crushed anaerobic granular sludge, obtained from a lab-scale UASB reactor treating synthetic brewery wastewater. The influent was similar to the above mentioned anaerobic medium in composition, except for the carbon sources. In this case, ethanol and organic acids, including propionate and acetate, served as the carbon sources with a total COD of 400 mg/L. The anode chamber and potentiostat were operated in the same way as in batch experiments. For comparison, a control anaerobic reactor was set up as well, which was not connected to the potentiostat. After a short start-up period of one week, the experimental period was divided into two phases: phase A (0–10 days) and phase B (10–19 days). The COD load in phase A remained the same as that in the start-up period (0.25 gCOD L⁻¹ d⁻¹), while it was doubled in phase B. The experimental systems were operated in the same way as the start-up period, except 40% of the medium was replaced in the cathode chambers on days 3, 8 and 14. The headspace gas and liquid from cathode chambers were sampled regularly for gas composition and COD determination. At the end of the experimental period, biofilms associated with cathodic electrodes were collected for protein quantitation and archaeal community analysis.
end of the experimental period, biofilm samples were collected from two reactors for protein quantitation and archaeal community analysis.

2.3. Analytical methods

H₂ was analyzed by gas chromatography (G1530A, Agilent) with a capillary column of 30.0 m × 0.53 mm (Carboxen® 1010 PLOT, Supelco). Helium was used as the carrier gas at a flow rate of 8.2 mL/min. The injection and detector temperatures were maintained at 225 °C. The column temperature was held at 40 °C for 15 min. For CH₄ and CO₂, a separate gas chromatograph (GC-8A, Shimadzu) was employed, which was equipped with a 2 m × 1/8” stainless column packed with HayeSep DB (100/120 mesh). Helium was the carrier gas at a flow rate of 21.4 mL/min. Other parameters were as follows: injection temperature of 110 °C, detector temperature of 110 °C, column of 50 °C held for 7 min. COD, ethanol and organic acids were measured by the previously described methods (Morita et al., 2011). Before protein measurement, the collected biofilm was dissolved in 20 mL isotonic wash buffer consisting of 4.19 g MOPS (morpholinepropanesulfonic acid), 0.60 g NaH₂PO₄, 0.10 g KCl, 5.00 g NaCl, and 10 mL Mg–Ca mixture (3.00 g/L MgSO₄·7H₂O, 0.10 g/L CaCl₂·2H₂O) per liter. The bicinchoninic acid method (Pierce, Rockford, IL, USA) was used to measure protein concentration with bovine serum albumin (BSA) as a standard. The 16S rRNA genes were amplified from genomic DNA by the polymerase chain reaction (PCR) using previously described primer set 344F/915R primer (Raskin et al., 1994). PCR products were purified with the Gel Extraction Kit (Qiagen, Valencia, CA, USA) and clone libraries were constructed with a TOPO TA cloning kit, version M (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions (Holmes et al., 2007). All plasmid inserts were sequenced with M13 forward and reverse primers and sequences were compared to GenBank nucleotide databases (Altschul et al., 1997).

2.4. Estimation of CH₄ production and coulombic efficiency (ηCE, %)

The theoretical production of CH₄ and CO₂ from degraded organic substrate was calculated as described previously (Angelidaki and Sanders, 2004). For practical purposes, 5% of the degraded organic substrate for biomass synthesis was considered. Coulombic efficiency (ηCE, %), the efficiency of electron transfer from the electrode to CO₂ to generate methane (Van Eerten-Jansen et al., 2012), was calculated on a daily basis with the following equation:

\[ \eta_{CE} = \frac{N_{CH_4, measured} - N_{CH_4, calculated}}{\int_0^T I dt / F} \cdot n \]  

where \( N_{CH_4, measured} \) is actual methane production (mmol CH₄), \( N_{CH_4, calculated} \) is estimated methane production (mmol CH₄), \( n \) is moles of electrons per mole of methane (8 mmol e⁻/mmol mmol CH₄), \( I \) is current (mA), \( t \) is time (s) and \( F \) is Faradays constant (96,485 mC/mmol e⁻).

2.5. Archaeal community analysis

The biofilm samples collected from electrodes were centrifuged and stored at −20 °C prior to community analysis. TE/sucrose DNA extraction protocol (Tender et al., 2002) was adopted for all of the samples. 16S rRNA genes were amplified from genomic DNA by the polymerase chain reaction (PCR) using previously described thermal cycler parameters (Holmes et al., 2004) with the archaeal specific primer set 344F/915R primer (Raskin et al., 1994). PCR products were purified with the Gel Extraction Kit (Qiagen, Valencia, CA, USA), and clone libraries were constructed with a TOPO TA cloning kit, version M (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions (Holmes et al., 2007). All plasmid inserts were sequenced with M13 forward and reverse primers and sequences were compared to GenBank nucleotide databases (Altschul et al., 1997).

3. Results and discussion

3.1. Performance of batch in-situ and ex-situ upgrading systems

Current density and biogas composition were monitored over the course of 19 days in both ex-situ and in-situ biogas upgrading systems to determine the efficiency of the bioelectrochemical method (Figs. 2a and 2b). Current density is directly related to CO₂ reduction rate in the cathode chamber (Villano et al., 2010; Van Eerten-Jansen et al., 2012). Therefore, it was used in this study to assess CO₂ reduction rate. As shown in Fig. 2a, current densities were significantly higher in the in-situ system, indicating that the design of the in-situ system allowed for better CO₂ reduction. The maximum current density of the in-situ system was around 1.0 A/m², compared to only 0.4 A/m² produced by the ex-situ system. The lower current densities observed in the ex-situ system were likely to result from CO₂ gas-liquid transfer limitations caused by the fact that CO₂ was produced in a separate digester and bubbled into the cathode chamber. These limitations could be greatly alleviated in the in-situ system because CO₂ production

Fig. 1b. Schematic of the integrated anaerobic reactor for biogas upgrading in continuous mode. The working (cathode), counter (anode) and reference electrodes were connected to a potentiostat and the cathode potential was poised at −700 mV (versus SHE). The working volume of the cathode chamber (digester) was 0.8 L.

Fig. 2a. Current density over time in ex-situ and in-situ biogas upgrading systems. The results shown here are data for a representative of duplicate systems. For phase A (0–10 days), the COD load for biogas upgrading systems was 0.25 gCOD L⁻¹ d⁻¹ (glucose). For phase B (10–19 days), the COD load was doubled. MR: medium replacement.
and reduction were able to take place simultaneously in the same chamber.

Spikes in current density were observed on a daily basis in both the in-situ and ex-situ systems and corresponded with glucose additions (Fig. 2a). It is likely that the added glucose was immediately decomposed into \( \text{CH}_4 \) and \( \text{CO}_2 \), and provided abundant electron acceptors (\( \text{CO}_2 \)) for methanogens, which was expected to accelerate the \( \text{CO}_2 \) reduction process and enhance current densities. Once all of the glucose was removed from the system and the available \( \text{CO}_2 \) started decreasing, current densities declined correspondingly. Further evidence that \( \text{CO}_2 \) reduction rate was correlated with \( \text{CO}_2 \) availability was provided during phase B of the experiment in both the in-situ and ex-situ systems. When COD load was doubled in phase B, nearly twice as much \( \text{CO}_2 \) was produced (data not shown), and current densities increased 30% and 100% in the in-situ and ex-situ systems (Fig. 2a). Medium replacement also appeared to enhance microbial activity and \( \text{CO}_2 \) reduction rate, for significantly larger spikes in current were seen on days 3, 8 and 14.

The composition of biogas from cathode chambers was compared to raw biogas produced by the anaerobic digester of the ex-situ system. As shown in Fig. 2b, \( \text{CO}_2 \) content in raw biogas was quite stable (~48%) throughout the experiment. The in-situ system was better than the ex-situ system at \( \text{CO}_2 \) removal in both phase A and B, which was consistent with results from current density measurements. In phase A, \( \text{CO}_2 \) content in biogas dropped below 10% in both systems. Although COD load was doubled in phase B, \( \text{CO}_2 \) content in the headspace of the cathode chamber remained low (~15%) (Fig. 2b). In addition, \( \text{H}_2 \) (up to 0.11%) was detected in both systems (Fig. 2b). It seems likely that \( \text{H}_2 \) production followed by hydrogenotrophic methanogenesis could be one of the major contributors to biogas upgrading in these systems.

Cathode performance of the H-cell devices in both ex-situ and in-situ biogas upgrading systems was evaluated on days 16–19 when \( \text{CO}_2 \) content had stabilized (Table 1). The contribution of electro- and/or hydrogenotrophic methanogenesis to total \( \text{CO}_2 \) reduction was determined by comparing total \( \text{CO}_2 \) removal to increased \( \text{CH}_4 \) production within the cathode chambers. If all of the \( \text{CO}_2 \) reduction in the systems was generated by electro- and/or hydrogenotrophic methanogenesis, total \( \text{CO}_2 \) removal and increased \( \text{CH}_4 \) values would be identical. However, increased \( \text{CH}_4 \) production in both the ex-situ and in-situ systems was consistently lower than the total amount of \( \text{CO}_2 \) removed from the systems (i.e. on day 16, around 0.95 and 1.05 mmol \( \text{CO}_2 \) were removed from the ex-situ and in-situ systems, but only 0.2 and 0.3 mmol more \( \text{CH}_4 \) were generated), even with coulombic efficiencies greater than 80%. In addition, more of the \( \text{CO}_2 \) removal could be attributed to electro- and/or hydrogenotrophic methanogenesis in the in-situ system than the ex-situ system; ca. 18.2% in the ex-situ system compared to 28.0% in the in-situ system.

A possible explanation for the discrepancy between total \( \text{CO}_2 \) removal and increased \( \text{CH}_4 \) production is that alkali products, which are effective at \( \text{CO}_2 \) absorption, were generated in the cathode chamber because many more cations (i.e. sodium and potassium) than protons were being transported across the membrane (Rabaey et al., 2010). Supporting evidence for this came from the fact that the pH of spent medium from the cathode chambers was 7.8–8.3, obviously higher than that of fresh medium (7.0 ± 0.1). Not only do these alkaline conditions increase \( \text{CO}_2 \) removal from the headspace, they also have the added benefit of reducing demand for external alkalinity addition and avoiding acidification in anaerobic digesters (Hawkes et al., 1994).

### 3.2. Performance of the integrated anaerobic reactor

The results from batch experiments conclusively showed that the in-situ biogas upgrading system was more efficient than the ex-situ one. To further facilitate its application, a new integrated anaerobic reactor run in continuous mode was implemented (Fig. 1b). Current density, \( \text{CO}_2 \) content and \( \text{CH}_4 \) production were monitored over time (Figs. 3a–c). During phase A of the experiment, current density gradually increased and stabilized at around 1.1 A/m² after 8 days (Fig. 3a). When the influent flow rate was increased from 250 to 500 mL/d in phase B of the experiment, current density increased significantly and the maximum current densities (up to 3 A/m²) were three times greater than those seen in batch systems.

The \( \text{CO}_2 \) content in raw biogas evolved from the continuous reactor (raw biogas values were determined in the control reactor) was significantly lower than the proportion of \( \text{CO}_2 \) found in raw biogas produced by glucose fed batch reactors; 25–30% (Fig. 3b) compared to 48% (Fig. 2b) \( \text{CO}_2 \) content. Different carbon sources were provided in these experiments; while glucose was added to the batch reactors, the continuous reactors were fed synthetic brewery wastewater which had a high concentration of ethanol.
and various organic acids (including propionate and acetate). Studies have shown that CO2 content in biogas produced from ethanol is significantly lower than CO2 associated with glucose generated biogas (Angelidaki and Sanders, 2004; Buswell and Neave, 1930).

In the experimental reactor, CO2 content gradually decreased over the course of phase A and reached its lowest point (∼8%) on day 10 when current densities stabilized (Figs. 3a and 3b). When COD levels were doubled during phase B of the experiment, CO2 content went up to 20% but gradually dropped back down to ∼14%. CO2 content in the control reactor also increased to 40% at the beginning of phase B, but stabilized at ∼30% after 5 days. Similar to results from the batch experiments, these results showed that even when COD levels were doubled during phase B of the continuous experiment, CO2 content in the experimental system continued to be significantly lower than the raw biogas found in the control reactor. In addition, the COD removal rate in the experimental reactor was consistently higher (∼11.3% in phase B) than that in the control reactor throughout the experiment.

Both CH4 production and coulombic efficiency (ηCE) of the experimental reactor were evaluated on days 16, 18, 20, 22 when CO2 content had stabilized (Fig. 3c). As demonstrated in Fig. 3c, the total CO2 amount removed from the experimental reactor was consistently higher than increased CH4 production. Similar results from the batch experiments, these results showed that besides the conversion of CO2 into CH4 by electro- and/or hydrogenotrophic methanogens, CO2 absorption by alkali products are likely to be involved in biogas upgrading. Coulombic efficiency was also similar to results from the batch reactors and ranged from 80–90%. Increased CH4 production in the continuous system was 3–4 times greater than that in the batch systems, indicating that bioelectrochemical reactions were responsible for a greater proportion of total CO2 removal than that of the batch systems.

3.3. Characterization of microbial colonization of biocathodes

Active biomass associated with biofilms that formed on the surface of biocathodes in the batch and continuous experiments was determined by protein measurements (Lanthier et al., 2008). In the batch experiments, cathode-associated biomass was 6 times greater in the in-situ biogas upgrading system (Fig. 4), which was consistent with greater CO2 reduction and current density observed in the in-situ system. It has already been shown that continuous feeding and hydraulic selection promotes biofilm formation (Nogueira et al., 2002). Therefore, it was not too surprising to find that significantly more protein was measured on electrodes recovered from continuous reactors than the batch systems (Fig. 4).
Approximately 10 and 2 times more protein was measured on the electrodes from the experimental continuous system than the ex-situ and in-situ batch systems. In addition, application of a poised cathode in the continuous system promoted biofilm formation; ca. 70% more protein was recovered from the experimental cathode compared to the control electrode.

The archaeal communities associated with various bioelectrode biofilms recovered from batch and continuous reactor systems were determined by 16S rRNA gene sequence analysis. Although different sources of sludge were used as inocula for the batch and continuous experiments, all of the bioelectrodes for biogas upgrading were dominated by the same hydrogenotrophic methanogen species (97–99% nucleotide sequence identity to Methanobacterium petrolearium) which accounted for ~50% of the total sequences in all 3 experimental conditions (Fig. 5). Sequences most similar to Methanoseta concilii (96–99% nucleotide sequence identity) also appeared to be enriched on the bioelectrode of both in-situ and ex-situ batch reactors, while sequences most similar to another hydrogenotrophic Methanobacterium species (94–99% similar to Methanobacterium subterraneum) were enriched in continuous mode. Overall, Methanobacterium was the most abundant genus in all three experimental conditions, which has also been seen in other published studies (Cheng et al., 2009; Marshall et al., 2012). While it is widely known that species from the genus Methanobacterium can use hydrogen as an electron donor for methanogenesis, its ability to extract electrons from the electrode needs further investigation. Species from the genus Methanoseta, on the other hand, have been shown to accept electrons through biological electrical connections for the reduction of CO2 to CH4 (Rotaru et al., 2014). Therefore, it seems possible that species from this genus could be engaged in electromethanogenesis by directly accepting electrons provided by the cathode. However, further investigation into this possibility is required.

4. Conclusions

Bioelectrochemical removal of CO2 for biogas upgrading was proposed here and demonstrated in both batch and continuous experiments. CO2 content in biogas could be kept below 10% in both the in-situ and ex-situ batch systems when the COD load was 0.25 gCOD L⁻¹ d⁻¹. The upgrading performance was further enhanced under continuous operation. Hydrogenotrophic methanogenesis and alkali production followed by CO2 absorption were likely to be major contributors to biogas upgrading in these systems. However, enrichment of species that have been shown to be capable of DIET on the batch cathodes indicated that electromethanogenesis could also be involved.

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