Microbial carbon capture cell using cyanobacteria for simultaneous power generation, carbon dioxide sequestration and wastewater treatment

Soumya Pandit, Bikram Kumar Nayak, Debabrata Das *

Department of Biotechnology, Indian Institute of Technology, Kharagpur, West Bengal, India

Article history:
Received 26 July 2011
Received in revised form 16 November 2011
Accepted 8 December 2011
Available online 21 December 2011

Keywords:
MCC
CO₂ sequestration
Flue gas
Anion exchange membrane
pH imbalance

Microbial carbon capture cells (MCCs) were constructed with cyanobacteria growing in a photo biocathode in dual-chambered flat plate mediator-less MFCs separated by an anion exchange membrane from the anode compartment containing Shewanella putrefaciens. The performance of the MCC with Anabaena sparged with CO₂–air mixture was compared with that of a conventional cathode sparged with air only. The power densities achieved were 57.8 mW/m² for Anabaena sparged with a CO₂–air mixture, 39.2 mW/m² for CO₂–air mixture sparging only, 29.7 mW/m² for Anabaena sparged with air, and 19.6 mW/m² for air sparging only. The pH of the cathode containing Anabaena gradually increased from 7 to 9.12 and power generation decreased from 34.7 to 23.8 mW/m² due to pH imbalance associated voltage losses without CO₂–air mixture sparging. Sparging with a 5% CO₂–air mixture produced maximum power of 100.1 mW/m². In addition, the power density of MCC increased by 31% when nitrate was added into the catholyte.

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1. Introduction

Microbial carbon capture cells (MCCs) are modified microbial fuel cells consisting of anode and cathode chambers separated by ion exchange membrane. Since the cathode chamber is sparged with CO₂, sequestration of this greenhouse gas can take place through biological conversion to organic matter by photosynthetic microorganisms such as microalgae and cyanobacteria. This organic matter can be transformed into products such as ethanol, biofertilizer, hydrogen and amino acids (González López et al., 2009). Wang et al. (2010) developed a MCC by introducing gas generated through bacterial respiration and metabolism at the anode into a cathode in which a photosynthetic microorganism (Chlorella vulgaris) was growing. They demonstrated the proof-of-concept of organic waste removal from the anode chamber with simultaneous electricity generation and carbon sequestration in the cathode chamber without the need of external energy inputs.

Cation exchange membranes (CEMs) have been commonly used in microbial fuel cells (MFCs) to separate the anodic and cathodic chambers (Rismani-Yazdi et al., 2008), but transport of cations other than protons through CEMs leads to pH imbalance-associated voltage loss and lower system stability and bioelectrochemical performance (Gil et al., 2003; Rozendal et al., 2006). Anion exchange membranes (AEMs) facilitate proton transfer by using phosphate or carbonate as the proton carrier and pH buffer (Kim et al., 2002; Rozendal et al., 2006; Gil et al., 2003), but a pH imbalance due to increase in catholyte pH may still occur. Therefore catholyte buffers play an important role in reduction reactions at the cathode by facilitating proton transfer through AEMs (Fan et al., 2007). Additionally, the buffers can stabilize the pH and increase conductivity thereby reducing the internal resistance of the MFCs. The use of bicarbonate buffer resulted in decrease in the internal resistance and an increased power density (Fan et al., 2007), and continuous addition of CO₂ to cathodes maintained sustainable catholyte pH and improved the anolyte pH, alkalinity and conductivity (Fornero et al., 2010).

In the present study, a flat panel MCC with a cathode chamber made similar to a flat panel reactor with high surface area/volume ratio was constructed. An open gas transfer area reduced the need for a dedicated degassing unit (Dasgupta et al., 2010). For sequestration of CO₂, Anabaena sp. was used since it has a high growth rate even in nitrate free medium (it can fix nitrogen from atmosphere) (González López et al., 2009). The performance of this MCC was compared to that of a conventional dual chamber MFCs in batch mode. In addition, the effect of different concentrations of CO₂ in CO₂–air mixtures, light and nitrate concentration in the catholyte was studied.

2. Methods

2.1. Microbial strain, media and growth conditions

Shewanella putrefaciens (ATCC BAA1097™) was maintained and grown in LB agar (composition: 10 g/L casein enzymic hydrolysate,
5 g/L yeast extract, 10 g/L NaCl, 15 g/L agar, final pH 7.5 ± 0.2) (HiMedia Laboratories Pvt. Ltd., India) at 37 °C. Anabaena strain. PCC 7120 was obtained from FotoMol, Uppsala University and pre-cultured in an illuminated autoclaved Erlenmeyer flask aerated with sterile air. The cyanobacterial cells were inoculated into 100-mL Erlenmeyer flasks containing 25 mL of the BG110 medium and kept in a shaking incubator at 30 °C and 150 rpm. One liter of the BG110 medium contained 0.04 g K2HPO4, 0.075 g MgSO4·7H2O, 0.036 g CaCl2·2H2O, 6.0 mg citric acid, 6.0 mg ferric ammonium citrate, 1.0 mg Na2EDTA, 0.02 g Na2CO3, and 1.0 mL trace metal solution A5. One liter of the trace metal solution A5 contained 2.86 g H2BO3, 1.81 g MnCl2·4 H2O, 0.222 g ZnSO4·7H2O, 0.39 g Na2MoO4·2H2O, 0.079 g CuSO4·5H2O, and 49.4 mg Co(NO3)2·6H2O. BG11 medium contained the same ingredients as in BG110, with 1.5 g/L NaNO3. A light intensity of 45 lEm/2 s/2 was applied using tube lights.

### 2.2. MCC assembly

4 flat plate dual-chambered MCCs were constructed using poly-acrylic sheet. Details of MCC setup are given in Table 1. The setup was assembled using steel studding, washers and nuts. Prior to use, graphite plate electrodes (32 cm² projected surface area) were washed with 1 N HCl followed by 1 N NaOH and thereafter soaked in deionised water for overnight. Copper wires were used for contact with electrodes and the contact portions were sealed with 'epoxy' material. Each chamber had provision for sample port, wire point inputs (top), reference electrode (Ag/AgCl, saturated KCl; +197 ± 2 mV, Equiptronics, India) inlet and outlet ports and anode chamber were sealed with washers to ensure anaerobic microenvironment, (Pandit et al., 2011). The cathode chamber was also configured in similar fashion with a lid on top to prevent evaporation of the catholyte.

### 2.3. MCC operation and experimental variations

The anode chamber was kept airtight and filled completely with 225 mL LB broth with the 25 mL overnight culture of 0.41 ± 0.01 g dry cell weight/l Shewanella putrefaciens (ATCC BAA 1097™) added to achieve anaerobic conditions in the least operation time. The catholyte for each MFC was prepared as per the experiment (Table 2). The MFCs were operated in both open circuit mode to let it reach maximum voltage as also in closed circuit mode using 1000 Ω. MCC and membrane can be sterilized by keeping the apparatus under UV exposure for at least 2 h followed by washing with 70% v/v aqueous ethanol solution gently, after use the membrane can be stored in Na2S2O5 aqueous solution of 0.1–0.5% v/w (Pandit et al., 2011).

### Table 1

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Anode chamber</th>
<th>Cathode chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Shewanella putrefaciens</td>
<td>Anabaena strain PCC 7120</td>
</tr>
<tr>
<td>Innoculum size (initial)</td>
<td>0.41 ± 0.01 g dry cell weight/l</td>
<td>0.2 g/l</td>
</tr>
<tr>
<td>Growth conditions</td>
<td>37 °C, 180 rpm, aerobic</td>
<td>30 ± 2 °C mixing with aeration</td>
</tr>
<tr>
<td>MCC reactor</td>
<td>Anode chamber</td>
<td>Cathode chamber</td>
</tr>
<tr>
<td>Dimensions (L × B × H)</td>
<td>10 cm × 10 cm × 2.5 cm</td>
<td>10 cm × 10 cm × 2.5 cm</td>
</tr>
<tr>
<td>Working volume</td>
<td>225 mL</td>
<td>225 mL</td>
</tr>
<tr>
<td>Operating mode</td>
<td>Anaerobic</td>
<td>Aerobic</td>
</tr>
<tr>
<td>Stirring conditions</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Gas, flow rate</td>
<td>None</td>
<td>3–7% (v/v) CO₂ enriched air, 200 mL/min</td>
</tr>
<tr>
<td>Initial pH</td>
<td>7.5</td>
<td>7.1</td>
</tr>
<tr>
<td>Electrodes</td>
<td>Anode</td>
<td>Cathode</td>
</tr>
<tr>
<td>Material</td>
<td>Noncatalyzed graphite plate</td>
<td>Noncatalyzed graphite plate</td>
</tr>
<tr>
<td>Projected surface area</td>
<td>32 cm²</td>
<td>32 cm²</td>
</tr>
<tr>
<td>Pretreatment</td>
<td>Sandpaper scrubbing, washing with 1 N HCl solution followed by 1 N NaOH solution</td>
<td>Sandpaper scrubbing, washing with 1 N HCl solution followed by 1 N NaOH solution</td>
</tr>
<tr>
<td>Electrode spacing</td>
<td>4 cm (anode to cathode)</td>
<td>4 cm (anode to cathode)</td>
</tr>
<tr>
<td>Wiring material</td>
<td>Concealed tin coated copper wire</td>
<td>Concealed tin coated copper wire</td>
</tr>
</tbody>
</table>

### Table 2

<table>
<thead>
<tr>
<th>Anolyte</th>
<th>Catholyte</th>
<th>Catholyte purged with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoclaved LB media, With inoculum of Shewanella putrefaciens</td>
<td>Autoclaved BG11 medium with inoculum of Anabaena strain PCC 7120</td>
<td>Air</td>
</tr>
<tr>
<td>Same as above</td>
<td>BG11 medium With inoculum of Anabaena strain PCC 7120</td>
<td>CO₂-air mixture 3% (v/v)CO₂ enriched air</td>
</tr>
<tr>
<td>Same as above</td>
<td>Distilled water</td>
<td>Air</td>
</tr>
<tr>
<td>Same as above</td>
<td>Distilled water</td>
<td>CO₂-air mixture 3% (v/v)CO₂ enriched air</td>
</tr>
</tbody>
</table>
For the biotic catholyte, *Anabaena Pcc 7120* was used and pre-cultured in an illuminated autoclaved Erlenmeyer flask aerated by sterile air. 50 mL (0.2 g/l) of actively growing culture of *Anabaena* sp. strain was inoculated to catholyte.

Biomass concentration was estimated by dry weight measurement. All estimations related to cell potentials, catholyte pH, cell biomass and chlorophyll content have been carried out in duplicate for each MCC setup. The pH was measured using a pH electrode with a sensitivity of 0.01 pH. The mixing of the cyanobacterial suspension in the cathode chamber was achieved by a gas sparger (Fig. 1). The sparger received gas from a gas mixer providing the desired relative concentrations of air and CO₂ (3% v/v) through filter of pore size 0.3 μm. The gas mixer assembly comprised of three rotameters (CM flow meters, India), connected with three metering valves for controlling the flow rates of each gas and three unidirectional valves.

2.4. **Analytical measurements and calculations**

The voltage between the anode and cathode and cathode with respect to the reference electrode were measured using a data acquisition system (USB-6009, National Instruments, Texas, USA) with NI LabVIEW–based customized software, Core Technologies, India). Polarization curves were obtained by varying the external resistance of the closed circuit using a variable resistance box (range 99999.9–0.1 Ω) in discrete steps and measuring the corresponding voltage drop. The average time required for obtaining a stable reading was 15 to 20 min. The current density, power density, and internal resistance were calculated previously described (Pandit et al., 2011). The internal resistance of the MCCs were calculated by the current interrupt method (Aelterman et al., 2006).

Internal resistance ($R_{\text{int}}$) of the MFC can be hence calculated as:

$$R_{\text{int}} = \frac{V_e}{I}$$  \hspace{1cm} (1)

The cyanobacteria concentrations were determined by measuring the optical density (OD) of the medium at 683 nm using a UV–visible spectrophotometer (Perkin Lambda 25 UV/VIS) along with calibration curves prepared for *Anabaena Pcc 7120* grown in BG11 media. Chlorophyll a content was determined using an extinction coefficient of 78.74 l g⁻¹ cm⁻¹ (Meeks and Castenholz, 1978).

The COD values of the anolyte were measured according to APHA standard methods (APHA, 1998) using a COD measurement instrument set (DRB200 & DR2800 Portable Spectrophotometer, HACH®, USA). The pH values were monitored using a desktop pH meter (pHS10, Cyberscan, Singapore). The light intensity was measured by a quantum sensor (Li-COR, Model Li-250A, Lambda Instrument Corporation, USA.)

3. Results and discussion

3.1. **Performance of MCC for electricity production using different catholyte**

Four flat plate MCCs were operated together in a batch mode. The details of the cathodic experimental conditions used in the MCCs are given in Table 2. In the abiotic state, the anodic half-cell potential was -189 ± 9 mV with respect to the Ag/AgCl reference electrode. The potential decreased to -481 ± 8 mV and stabilized after 7 h. The minimum anode potential reached -493 mV vs. Ag/AgCl 9 h after inoculation similar to that what was reported by Kim et al. (2002). Significant variation was found in the cathode half-cell potential. For each of the setups, the cathodic potential developed slowly for 14–18 h from start-up and then stabilized at 129 ± 7 and 108 ± 9 mV for CO₂–air mixture sparging and air sparging, respectively. However, it was noted that the cathodic half cell–potential dropped after 40–45 h for the set up sparged with air while the potential remained constant for the set up sparged with CO₂–air mixture. The pH gradually increased towards alkalinity leading to pH splitting which decreased the overall open circuit potential (OCP). This may be attributed to the fact that *Anabaena* may be withdrawing carbonate/bicarbonate from the catholyte. However, a steady OCP of around 717 ± 9 mV using 3% CO₂–air mixture after 26 ± 3 h from start-up for the *Anabaena* grown cathode was observed (Table 2). This result indicated that continuous sparging a CO₂–air mixture into the *Anabaena*-containing catholyte facilitated maintaining the cathodic pH at nearly 7 (Figs. 2a–2c). CO₂ dissolved in the catholyte and showed buffering action. As a result, the pH imbalance between anode and cathode chamber was prevented. The cell concentration also increased continuously and consequently the dissolved oxygen in catholyte increased, too. Under alkaline conditions, CO₂ dissolves easily aiding biomass production (Wang et al., 2010). Hence a steady OCP of around 717 ± 9 mV was documented using a 3% CO₂–air mixture in the *Anabaena*-containing MCC.

Once the MCCs reached steady maxima in their OCP, polarization studies were performed. The corresponding polarization curves of the MCCs (Fig. 2d) were obtained by varying the external resistance from 10 Ω to 90 kΩ. The power density of MCCs was in the following order: set-up with *Anabaena* with CO₂–air mixture sparging > CO₂–air mixture sparging > *Anabaena* with air sparging > air sparging. An increase of 61% in power density (from...
22.51 to 57.8 mW/m²) could be achieved in the catholyte with Anabaena and sparged with a 3% CO₂–air mixture as compared to air sparging without Anabaena in catholyte. This outcome may be attributed to the buffering action (Fornero et al., 2010) by CO₂ as it dissolves to form carbonate/bicarbonate and/or CO₂ and N₂ reduction in the cathode chamber as cell biomass is produced. Since O₂ is the primary electron acceptor, saturated oxygen levels generated by passive aeration provided to the cathode by the photosynthetic activity of Anabaena may have further improved power generation. Some of the inorganic carbon species (IC) were reduced to cell mass. At pH 7.0, CO₂ reduction has a low redox potential of −0.42 V (vs. standard hydrogen electrode (SHE), thus its use in a MCC may result in low voltage production; however, it has been shown previously that light facilitates CO₂ reduction at the cathode in a MCC (Cao et al., 2009). Nitrogen from the air was reduced to ammonium and assimilated by Anabaena (Lindblad et al., 2002).

Further, it was found that 17 ± 8% more anolyte COD reduced during closed circuit (100 Ω) mode batch operation in MCC compare to conventional dual chambered MFC. This fact signifies the suitability of MCC for wastewater treatment.

3.2. Effect of CO₂ concentration in CO₂–air mixture

The effect of 1–7% CO₂ concentration in CO₂–air mixture mimicking industrial flue gas composition on power generation was investigated. The performance of MCC was found highest in terms of power density at 3% CO₂–air sparging. The polarization studies shown in Fig. 3a indicate that the performance of MCC was found to be better at 3% CO₂–air sparging than at other CO₂–air mixtures (1%, 5% and 7% v/v) sparging in cathode chamber.

Fig. 2b. Cathodic half-cell potentials of microbial carbon capture cells (MCCs) using Anabaena with -air sparging in catholyte.

Fig. 2c. Cathodic half-cell potentials of microbial carbon capture cells (MCCs) using Anabaena with 3% CO₂-air sparging in catholyte.

Fig. 2d. Comparison of polarization studies of microbial carbon capture cells (MCCs) using Anabaena with CO₂–air mixture sparging, CO₂–air mixture sparging, Anabaena with air sparging and only air sparging in catholyte.

Fig. 3a. Comparison of polarization studies of microbial carbon capture cells (MCCs) using different CO₂–air mixture (1%, 3%, 5% and 7% v/v) sparging in cathode chamber.

Fig. 3b. Profile of cell biomass concentration and chlorophyll concentration in microbial carbon capture cells (MCCs) using different CO₂–air mixture (1%, 3%, 5% and 7% v/v) sparging in cathode chamber.
of power generation, when the cathode chamber was sparged with a 5% (v/v) CO₂-air mixture. An increase of 58.7% in power density was found from 41.3 to 100.13 mW/m² when the CO₂ concentration was increased from 1% to 5%; however, any further increase in CO₂ concentration had no significant effect on power density. When the cathode chamber was sparged with a 7% (v/v) CO₂-air mixture, power density obtained was 97.9 mW/m² (Fig. 3a). The increase in power output may be attributed to the cumulative effect of increased dissolve O₂ concentration due to biophotolysis of *Anabaena*, buffering action of CO₂/bicarbonate as better proton compensation through the continuous flow of CO₂-air mixture, and bicarbonate reduction to cell biomass at the cathode (Eq. (2)). As shown in Figs. 3b and 3c a gradual increase in cell biomass as well as chlorophyll content was observed suggesting that enhanced biophotolysis in the presence of light led to increased dissolved oxygen concentrations (Wang et al., 2010): 

\[ n\text{CO}_2 + n\text{H}_2\text{O} \rightarrow \text{CH}_2\text{O} \text{H}_n + n\text{O}_2 \]  

(2)

Previously it was reported that the power output of two-chambered MFCs was proportional to the concentration of dissolved O₂ in the catholyte (Rismani-Yazdi et al., 2008). An improved MCC configuration may further boost power output by improving the CO₂ mixing in the cathode chamber. Hence, flue gas may be considered as a viable option for improving the buffering capacity. Further, it may be sequestered by photosynthetic bacteria in catholyte chamber which may further enhance power generation.

### 3.3. Effect of nitrate

The effect of nitrate was observed by feeding BG11 instead of BG11o medium to the cathode compartment along with continuous CO₂-air mixture sparging. The maximum sustainable power density of the MCC increased from 52.8 to 76 mW m⁻² (Table 3). Increment in power may be due to the addition of the electron acceptor, nitrate, to the catholyte. Enhanced power generation through nitrate supplementation to catholyte in MFC is already reported by Behera et al. (2010) and Rodrigo et al. (2010). Nitrate may behave as an alternative to oxygen as electron-acceptors in the cathodic chamber. The nitrate uptake rate (NUR) was equal to the oxygen uptake rate. Under phototrophic conditions, cyanobacteria reduce nitrate to ammonium by nitrate reducase and N₂ to ammonium by nitrogenase. Flue gas containing NOx forms nitrate in solution which can act as additional electron acceptor (Cheremisinoff, 2001; Peters and Holman, 1955).

Results obtained in terms of power generation have been compared to other relevant studies (Table 4). The results indicate that operating MCC by introducing flue gas could be a viable option for CO₂ sequestration and energy recovery while treating wastewater. MCC could be a potential integrated wastewater treatment tool for industries which simultaneously discharge polluted water and emit CO₂.

### 3.4. Effect of light

A gradual decrease in current was observed as soon as the light source was removed. When light was restored to the cathode, voltage generation resumed and increased over time (Fig. 4). This indicated that voltage generation depends on both light and bicarbonate utilization. In the dark phase from 3 h and from 9 h, cell voltage gradually decreased and reached a plateau of about

### Table 3

The maximum open circuit potential and power densities of the MCCs using BG11 and BG11o medium in catholyte.

<table>
<thead>
<tr>
<th>Type of culture medium</th>
<th>Power density (mW/m²)</th>
<th>Voltage (OCV in mV)</th>
<th>Current density (mA/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BG11</td>
<td>76.05</td>
<td>734</td>
<td>487.5</td>
</tr>
<tr>
<td>BG11o</td>
<td>52.81</td>
<td>698</td>
<td>406.5</td>
</tr>
</tbody>
</table>

### Table 4

Comparison of experimental parameters and highest power densities achieved with different experimental systems.

<table>
<thead>
<tr>
<th>Anolyte volume (mL)</th>
<th>Anode</th>
<th>Cathode</th>
<th>Highest power density</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>Plain graphite felt (8 cm²)</td>
<td>Treated graphite felt (8 cm²)</td>
<td>750 mW/m²</td>
<td>Cao et al. (2009)</td>
</tr>
<tr>
<td>220</td>
<td>Carbon fiber brushes</td>
<td>Platinised carbon cloth (48 cm²)</td>
<td>5.6 W/m²</td>
<td>Wang et al. (2010)</td>
</tr>
<tr>
<td>225</td>
<td>Uncoated graphite plate</td>
<td>Uncoated graphite plate</td>
<td>73.5 mW/m²</td>
<td>This study</td>
</tr>
<tr>
<td>300</td>
<td>Graphite (167 cm²)</td>
<td>ss-mesh (100 cm²)</td>
<td>20.3 mW/m²</td>
<td>Behera et al. (2010)</td>
</tr>
<tr>
<td>100</td>
<td>Glassy graphite rod</td>
<td>Glassy graphite rod</td>
<td>Not available, power ≤ 41 μW</td>
<td>Powell et al. (2009)</td>
</tr>
</tbody>
</table>
68 ± 5 mV vs. 100 Ω Rext; however, when light was restored at 45 μM s⁻¹ from 3 to 6 h and 9 h onwards, the voltage regained its earlier value as shown in Fig. 4. This finding indicates that light intensity may be critical to the stability of the MCC as it affects O2 generation through biophotolysis. Reduction of oxygen in the cathode is a rate limiting step; without illumination, oxygen generation through biophotolysis might stop (Cao et al., 2009). When the MCC was kept under prolonged darkness (Fig. 4), the voltage dropped drastically. It was also noticed that the cyanobacterial cell biomass concentrations were almost same (0.2 ± 0.01 g/L) for the initial (0 h), intermediate (7 h) and final (14 h) samples collected from the catholyte. This result indicates that bicarbonate reduction as well as biophotolysis ceased in the absence of light. Restoration of illumination improved cell performance but not to its initial level.

4. Conclusion

MCCs were explored for integrated wastewater treatment, CO2 sequestration and electricity generation. The power outputs of MCCs were better than those of dual-chambered MFCs. Flue gas which contains large amounts of CO2 might provide suitable buffering capacity and high NO3- containing flue gas could be used for power augmentation since nitrate can act as potential electron acceptor. Power generation in a MCC depends on both light and bicarbonate utilization. Factors such as light intensity, superficial gas velocity, reactor design need to be optimized for maximizing power generation in MCCs.

Acknowledgements

The authors wish to thank the financial support from Bhabha Atomic Research Centre (BARC), UGC, MNRE and DBT, Govt of India and IIT Kharagpur for the facilities.

References


