Potential of lignin as a mediator in combined systems for biomethane and electricity production from ethanol stillage wastewater

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Article info
Article history:
Received 26 December 2013
Accepted 5 November 2014
Available online

Keywords:
Two-compartment microbial fuel cell
Proton exchange membrane
Salt bridge
Mediator
Ethanol stillage wastewater
Electrical power generation

Abstract
This study investigated the feasibility of using lignin waste from pulp and paper industry as a mediator for treating ethanol stillage wastewater in microbial fuel cells (MFCs). Two configurations of MFCs used a salt-bridge and proton exchange membrane (PEM), and the influence of lignin and KMnO4 as mediators in PEM MFCs was evaluated. For salt-bridge MFC, graphite plates were used as cathode and anode while carbon cloth and graphite rods were substituted for cathode and anode in PEM MFC. The external resistance for both configurations was 550 ± 50 Ω. Results showed that PEM MFC generated higher electric power with satisfactory treatment of wastewater. When lignin was added as a mediator in PEM MFC, the maximum power density increased to 93 W/m² with COD removal efficiency of about 81% after 6-day operation. This study showed the potential for the treatment of lignin-rich wastewater and ethanol stillage wastewater simultaneously with possibility of electric power generation.

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1. Introduction

A microbial fuel cell (MFC) is a fuel cell which produces electrical energy from organic wastes through a bioelectrochemical reaction using bacteria. During anaerobic digestion processes of wastewater, the organic matter is decomposed, and electricity and biogas are generated [1,2]. One potential application of this kind of fuel cell is the treatment of wastewater effluents containing a variety of organic matters. Anaerobic digestion has been utilized to reduce organic content in wastewater in terms of biological oxygen demand (BOD) and chemical oxygen demand (COD) [3]. The growth of microorganisms that decompose organic matter results in the release of protons and electrons at the anode electrode. A typical MFC consists of an anode and a cathode separated by electrolyte proton transfer system. In MFCs system, the electrons move from the cathode to the anode electrode through the external circuit. The protons move from the anode electrode through the proton transfer system to the cathode and react with oxygen to produce water [4]. Therefore, the circuit is closed and electricity is produced.

MFCs can be divided into two types according to their configurations: 1) single-chamber MFC and 2) two-chamber MFC. For a single-chamber MFC, it consists of one compartment for anode in which the electrochemical reaction takes place while cathode is exposed to the air. The protons transfer from the electrolyte solution inside the anode compartment to the cathode attached to the proton exchange membrane (PEM). Both PEM and cathode are installed outside of the anode chamber as oxygen is required during electron acceptance phenomenon. In the case of two-chamber MFC, it is composed of anode and cathode compartments separated by a proton exchange system as shown in Figs. 1 and 2. The protons generated in anode compartment transfer through either the salt bridge or PEM to react with oxygen and electrons transferred from anode-to-cathode chamber via conductive wire to finally form water molecules. Limitations of low oxygen dissolution in catholyte to react on cathode surface can be overcome by sparging air through the catholyte [5,6] and adding various sorts of electron acceptors e.g. hexacyanoferrate, hydrogen peroxide, potassium permanganate (KMnO4), potassium ferricyanide (K3[Fe(CN)6]) and potassium dichromate (K2Cr2O7), etc.
Among all cathodic electron acceptors tested, it has been demonstrated that KMnO4 can be very effective in enhancing the power output of MFCs [8]. Additionally, mediators such as 9,10-anthraquinone-2,6-disulfonic acid disodium salt (AQDS), safranine O, resazurin, methylene blue, and humic acids have been added into the anode compartment [9,10] to enhance the electron transfer between exo-electrogens or chemical reaction and the anode surface. Although single-chamber MFCs show better performance than two-chamber MFCs, their use requires a coating of a precious metal such as platinum on the cathode surface to catalyze the electron accepting reaction at low temperatures close to room conditions [5]. Moreover, the method using carbon cathode coated with metal catalysts is relatively complicated with limited durability [11]. In cathodic compartment of two-chamber MFCs, electron acceptors can be used to facilitate the electron acceptance reaction, thus the coating by the costly metals can be omitted. Another advantage is that two chamber MFCs can easily operate in either batch or continuous modes.

The high cost of selective PEM in fabricating the MFCs has limited their utilization only in the laboratory scale studies. Many attempts have been made to study the influence of various MFC configurations on their performance. The influence of reactor configuration e.g. tubular, stacked, fixed-bed and fluidized bed as well as different proton transfer systems, types of cathodes and anodes have been investigated to maximize the performance of the designed MFCs [4,12].

The main objective of this study was to investigate the treatment of ethanol stillage wastewater using two-compartment MFCs with different configurations consisting of a salt-bridge and proton exchange membrane (PEM). Potassium permanganate (KMnO4) was used as electron acceptor in cathodic compartment without any high cost catalyst. In addition, a secondary objective was to evaluate the potential of using lignin waste from the pulp and paper industry as a mediator for power generation in the MFCs and its influence on the treatment efficiency of the ethanol stillage wastewater.

2. Materials and methods

Ethanol stillage wastewater (4% total solids) and anaerobic digesting sludge (9% total solids) were supplied from an ethanol production plant in Nakorn Pathom province, Thailand. Alkali lignin with low sulfonate content and approximate molecular weight of 10,000 g mol\(^{-1}\) was procured from Sigma–Aldrich. KCl, NaOH, NH\(_4\)Cl, K\(_2\)HPO\(_4\), MgSO\(_4\)·7H\(_2\)O, yeast extract, KMnO4, methylene blue and other analytical grade chemicals were purchased from Sigma–Aldrich, Merck, and Ajax Finechem. The proton exchange membrane (Nafion 117) was from Dupont, USA and carbon cloth was contributed by Asia Kangnum Co., Ltd., Thailand.

2.1. Influence of mediator on microbial growth and biogas production

For batch tests, 350 mL anaerobic digesting sludge inoculum was mixed with 350 mL nutrient solution containing 0.03 g L\(^{-1}\) NH\(_4\)Cl, 0.2 g L\(^{-1}\) K\(_2\)HPO\(_4\), 0.05 g L\(^{-1}\) MgSO\(_4\)·7H\(_2\)O, and 0.01 g L\(^{-1}\) yeast extract [13]. Anaerobic digestion was performed in a 1000-mL batch reactor. Then, 0.1 mol L\(^{-1}\) of potassium permanganate, lignin and methylene blue was added separately in each reactor to investigate the inhibiting effect of mediators on microbial growth and biogas production. After flushing reactor’s headspace with nitrogen gas, anaerobic digestion was carried out at 37 °C for 14 days. Cumulative biogas production was measured by water displacement method and followed by the determination of biogas composition. The viable cell count was determined following the assay. The biogas production was recorded when using different mediators in the system.

2.2. Anaerobic wastewater treatment in MFC

Anaerobic digestion in the anodic chamber was carried out using equal proportion of sludge and stillage wastewater by
volume. The digestion was performed in a 1000-ml Duran flask using 700 ml fermentation medium. The initial pH of the fermentation medium was adjusted to 7.2 and temperature was controlled at 37 °C. The digestion was stopped when no further increase in the biogas production was observed approximately in 7–14 days. A best performing mediator was selected from the screening experiments described in the previous section and added (0.1 mol L⁻¹) in the anodic compartment. The wastewater was sampled during digestion in the reactor to determine the pH and COD. Cumulative biogas generation and cell voltage were recorded every 4–6 h. The performance of MFCs was expressed in terms of the power density, biogas production and wastewater treatment efficiency.

2.3. Configuration of microbial fuel cells (MFCs)

2.3.1. Salt-bridge microbial fuel cells (salt-bridge MFC)

The salt-bridge MFC used in the experiment consisted of two separate anaerobic and aerobic compartments connected together by a salt bridge containing 1 mol L⁻¹ KCl agar as shown in Fig. 1. The salt bridge was 1-cm in diameter with cross sectional area of 0.79 cm² and axial distance of 10 cm. Both aerobic and anaerobic compartments had 1-L capacity and were equipped with graphite plate electrodes (2.5 cm × 7.0 cm × 0.35 cm) and conducting wires. A digital multi-meter was connected to monitor the voltage across a 500 Ω external resistor during the digestion period. The anaerobic chamber contained 700 mL of ethanol stillage wastewater with initial COD approximately in 35,000–38,000 mg L⁻¹ range. The chamber was flushed with nitrogen gas for 5 min before starting up the system in order to maintain anaerobic conditions. During digestion, the solution was continuously stirred using a magnetic stirrer at 300 rpm.

2.3.2. Proton exchange membrane microbial fuel cell (PEM MFC)

As illustrated in Fig. 2, 4-L PEM MFC consisted of anodic and cathodic chambers. The anodic chamber was cylindrical (12-cm diameter and 32-cm height) and made of borosilicate glass. The cathodic chamber had 500-mL capacity and was positioned above the anodic chamber. These two chambers were separated by a PEM (Nafton 117, Dupont, USA) [14] 12-cm in diameter with a cross sectional area of 113.1 cm². The PEM was held by a clamp between the flattened end of the vertical cylindrical reactor and the cover of the cathodic chamber. The distance between the cathode and anode was 11.5 cm. The interface between the cathodic and anodic compartments was sealed with an air tight gasket and equipped with carbon cloth (12-cm diameter and 0.1-cm thickness) as a cathode. The anodes consisted of the graphite rods penetrating into the bed of granular activated carbon weighing 300 g. Copper wires were used to connect the electrodes and an external resistor with a multi-meter. The volume of biogas produced from the system was measured by a water displacement apparatus connected to the gas outlet port. Ethanol stillage wastewater with initial COD of 35,000–38,000 mg L⁻¹ was pumped into the MFCs from the bottom of the anodic chamber and recirculated in the system at the flow rate of 24 mL min⁻¹.
2.4. Related measurements

Cumulative biogas production was recorded over the digesting period. The composition of biogas samples was analyzed by a gas chromatograph connected to a thermal conductivity detector (GC-TCD, SHIMADZU GC-14B, Japan). Chemical oxygen demand (COD) was determined from the wastewater samples over the treatment duration [13,15]. Total solids and volatile solids were measured according to the standard methods [16]. Cell voltage (V) was monitored over a 500 Ω external resistance. Power was calculated as $P = V^2/R$ and expressed as power density in W/m² based on the total anode surface area. The power was normalized as W/m³ based on the volume of wastewater [12,14].

3. Results and discussion

3.1. Influence of mediator on microbial growth and biogas production in batch reactor

Experiments were conducted to investigate the effect of mediator on microbial growth and biogas production utilizing glucose as a substrate as well as the digesting sludge from an anaerobic biogas plant as the starting inoculum. Results in Table 1 show the influence of mediators apparently had some effect on biogas producing bacteria which is in agreement with the findings of Wei and coworkers [13]. Table 2 presents the results of using lignin and methylene blue mediators in MFCs on the methane, carbon dioxide, and total biogas production on the 8th and 10th day of digestion. The difference between the volumes of methane and carbon dioxide and total volume of biogas indicated the volume of hydrogen gas produced. Methylene blue acted most likely as a cytotoxic mediator since there was no biogas production in its presence. Lignin had little effect on the production of methane. Total biogas production from the system containing lignin was less than that of the control on the 10th day. The volume of methane generated in 10 days was slightly in presence of lignin (162.8 mL) compared to the system without lignin (170.9 mL). Nevertheless, on the 10th day, methane production was significantly higher in presence of lignin (67.8% v/v) compared to the control system without addition of mediator (51.8% v/v). Moreover, the differences in the volumes of methane in systems with and without lignin were only 5 and 8 mL on the 8th and 10th day of digestion, respectively. Although, there have been reports on the effect of mediators on alteration of the fermentation process, an overall improvement resulted in the electrical current in MFCs by most mediators [9,10,18]. In the present study, lignin was least toxic to the microorganisms and had only small effect on the biogas generation. In addition, lignin molecules are analogous to humic acid which has been previously reported as a good mediator for bioelectricity production in MFC [9,19]. Thus, lignin was selected as a mediator in subsequent experiments to enhance power output in MFCs.

3.2. Performance of two-compartment salt bridge and PEM MFCs

Ethanol stillage wastewater was treated in MFC with equal proportion of sludge by volume at 37 °C and initial pH of 7.2. As shown in Fig. 4, cell voltage in case of salt bridge MFC and PEM MFC

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Digestion period (days)</th>
<th>Initial cell count (cell mL⁻¹)</th>
<th>Final cell count (cell mL⁻¹)</th>
<th>Volatile solids consumed (mg g⁻¹)</th>
<th>Cumulative gas volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sludge</td>
<td>25</td>
<td>$3.56 \times 10^6$</td>
<td>$2.28 \times 10^6$</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Sludge + Glucose</td>
<td>25</td>
<td>$2.80 \times 10^6$</td>
<td>$9.67 \times 10^6$</td>
<td>3.21</td>
<td>400</td>
</tr>
<tr>
<td>Sludge + Glucose + Lignin</td>
<td>25</td>
<td>$2.50 \times 10^6$</td>
<td>$7.74 \times 10^6$</td>
<td>1.06</td>
<td>356</td>
</tr>
<tr>
<td>Sludge + Glucose + KMnO₄</td>
<td>13</td>
<td>$3.10 \times 10^6$</td>
<td>$6.40 \times 10^6$</td>
<td>n.d.</td>
<td>160</td>
</tr>
<tr>
<td>Sludge + Glucose + Methylene blue</td>
<td>13</td>
<td>$2.70 \times 10^6$</td>
<td>$1.50 \times 10^6$</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

n.d. – not detectable.
had similar profiles over the digestion period of 14 days. The changes in cell voltage indicated the degradation of organic matters in the wastewater by the microorganisms corresponding to the growth phase [4,12]. As a result, the lag phase of microbial growth in the wastewater by the microorganisms corresponding to the changes in cell voltage indicated the degradation of organic matters.

Table 2 presents maximum power generation and COD removal by the salt-bridge and PEM MFCs. The maximum power density in PEM MFC based on the anode surface area (0.15 W/m^2) was significantly higher than in salt-bridge MFC (0.11 W/m^2). These values of maximum power density were much higher than 2.2 mW/m^2 (Geobacter metallireducens) for salt bridge MFC and 38 mW/m^2 (wastewater inoculums) for PEM MFC reported by Min and coworkers [21]. The COD removal efficiency of wastewater treatment in the experimental MFCs was about 70%–73%. However, the volume of the ethanol stillage wastewater treated in PEM MFC was 4.5 times the volume in the salt-bridge MFC for approximately similar levels of COD removal efficiency. Previous reports indicate that both salt bridge [8,21] and PEM [22,23] have been used as proton transfer system in MFCs. In general, the salt-bridge MFCs produce much less power output compared to the PEM MFCs. The low power output in salt-bridge MFCs has been directly attributed to the higher internal resistance compared with that of the membrane system based on the measurements using impedance spectroscopy. Min and coworkers demonstrated that internal resistance of salt-bridge MFC (19.9 kΩ) was 15 times higher than that of PEM MFC (1.3 kΩ) [21].

Therefore, PEM MFC had advantages over the salt-bridge MFC in terms of electrical power generation at the similar level of COD removal efficiency. Moreover, the wastewater can be continuously fed into the PEM MFC similar to an up-flow anaerobic sludge blanket (UASB) conventional bioreactor used for the anaerobic wastewater treatment in many industries. Thus, the development of PEM MFCs has potential and feasibility for industrial applications.

3.3. Influence of lignin on power generation in PEM MFC

It has been reported that lignin derived compounds were employed as redox mediators for enhancing the activity of lignin-degrading enzymes [24,25]. Due to its resistance to digestion by the microorganisms, it was proposed that lignin possibly caused inhibiting effect on the methanogens in mixed culture anaerobic digestion [26,27], thus enhancing the exoelectrogen activity for bioelectrical generation.

The addition of lignin in anodic chamber of so called lignin-mediated MFC resulted in substantial increase in the power generation based on the electrode area as well as wastewater volume of PEM MFC in comparison with the mediatorless MFC (Table 4). The COD removal from the wastewater was increased from 70% to
81%. These results were in good agreement with the previously reported work stating that the presence of humic acid in digested manure wastewater or lignocellulose material pretreated anaerobically or by physical–chemical methods such as wet oxidation enhanced the power generation. However, there was no evidence of the influence of humic acid ionic strength on power generation when KCl was used as a control. According to the theory, the maximum power output is obtained when the external and internal resistances are equal [29]. The internal resistance was lowered due to the formation of the humic acid [19]. As lignin is one of the major constituents in lignocelluloses [13] having molecular structure similar to the humic acid, it is believed to have the similar effect on the internal resistance and power output in MFCs. Thus, such systems have potential for the treatment of lignin-rich wastewater together in MFCs while generating the electricity as a byproduct.

3.4. Scanning electron microscope (SEM) images of electrodes

The SEM images of the microorganisms attached onto the surfaces of both graphite rods and carbon cloth following their use as electrodes in the MFCs are shown in Fig. 5(a) and (b), respectively. The biofilm formed by the microorganisms on the surface of graphite rods was thicker compared to the carbon cloth indicating the graphite to be a good electrode material for the MFCs. The carbon cloth used in the present study was relatively glossy and hydrophobic. Although the formation of biofilm significantly increases the electron transfer rate from the microorganisms to the anode surface, the thickness of the biofilm may adversely affect the internal resistance of the system and thus decrease the power output [30]. Consequently, the optimization of biofilm thickness on the electrodes has been widely studied for the several configurations of the MFCs [31,32]. In addition, metal foams and porous materials such as activated carbon [33] and nickel foam [34] have been utilized recently as electrodes in MFCs to facilitate electron transfer from microbial cells to electrode surface and thus increasing the power generation. It has been revealed that an increase in the number of pores during the formation of colonies by the microbes leads to higher power density produced from the MFCs. Therefore, the development of novel materials for electrodes having good morphology and conductivity is a challenging area of research worldwide.

4. Conclusions

The performance of proton exchange membrane microbial fuel cell (PEM MFC) was superior compared with the salt-bridge microbial fuel cell in terms of the power density generation for approximately similar levels of COD removal from the ethanol stillage wastewater. Lignin was a good mediator having less toxicity than methylene blue and KMnO4 on the microbial growth and considerably enhanced power output from the PEM MFC for wastewater treatment. Consequently, PEM MFC was a promising system for the treatment of effluent from the pulp and paper industries or other related processes such as the treatment of recalcitrant dyes. In order to achieve maximum performance of a given MFC configuration, the parameters such as bacterial strains, external resistance, cathode and anode are required to be optimized. It was feasible to treat both lignin waste and ethanol stillage wastewater together in MFCs while generating the electricity as a byproduct.

Acknowledgment

This work was financially supported by the grant from the Energy Policy and Planning Office (EPPO), Ministry of Energy, Bangkok, Thailand (Fiscal year 2011).

References


