ABSTRACT

YAN, DI. Electricity Generation and H₂ Evolution by Microbial Fuel Cells. (Under the direction of Dr. Wenqiao Yuan.)

The microbial fuel cell (MFC) has gained much attention because of its ability to generate power from organic or inorganic compounds via microorganisms. Based upon the ability of converting chemical energy to electrical energy, MFCs have many promising applications, such as electricity generation, bio-hydrogen production, wastewater treatment and biosensor. Hydrogen is one of the most important future fuels because it is recognized as an ideal renewable energy source with less greenhouse gas production. However, several challenges lie in hydrogen production processes, such as the high cost and the by-products in production processes from coal or natural gas. Biological processes for H₂ production can achieve a higher yield with the lower cost of materials and transportation. Microbial electrolysis cell (MEC) is a newly developed technology based on the concept of MFC for bio-hydrogen production, which combines bacterial metabolism with electrochemistry. For H₂ gas evolution, MECs are found to be advantageous to the fermentation due to its ability to overcome the thermodynamic barrier dealing with fermentation byproducts, such as acetate or butyrate. In this project, a two-stage process combining dark fermentation with MFCs/MECs was developed for electricity generation and H₂ production from recalcitrant lignocellulosic materials. This research focused on evaluating the performance of MFCs for electricity generation and H₂ production by feeding with sodium acetate and fermentation product.
The first objective of this study was to explore the effects of several operational parameters on the performance of MFCs for electricity generation and H₂ production. The effects of substrate concentration, electrode surface area, medium conductivity, membranes and applied voltage were investigated. It was found that *Geobacter sulfurreducens* (ATCC 51573) could produce electricity and hydrogen gas in MFCs by degrading acetate as the electron donor and using carbon cloth as sole electron acceptor. The different types of membranes impacted the performance of MFCs and MECs: cation exchange membrane (CEM) was advantageous over proton exchange membrane (PEM) for power generation, and PEMs outperformed CEMs for hydrogen gas production by MECs. In addition, the performance of MFCs and MECs was strongly related to substrate concentration, electrode surface area, medium conductivity and applied voltage.

The second objective was to evaluate the performance of MFCs for electricity generation and H₂ evolution by using the acetic acid from anaerobic fermentation as feedstock. It was demonstrated that *Moorella thermoacetica* (ATCC 49707) could degrade corncob hydrolysate as a primary substrate with a high yield of acetic acid through dark-fermentation. The fermentation product, acetic acid, could be used as a substrate by *G. sulfurreducens* in MFCs electricity generation and H₂ production. In the MFCs/MECs fed by fermentation broth, using an anodic electrode colonized by *G. sulfurreducens* had better performance for both generation and H₂ evolution.
Electricity Generation and H₂ Evolution by Microbial Fuel Cells

by
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DEDICATION

This work is dedicated to my parents, Mr. Xiaoxi Yan and Mrs. Qiuyue Wang, and my boyfriend, Mr. Yupeng Liu, for their endless financial and emotional support to all the way through.
BIOGRAPHY

Di Yan was born in Langfang, Hebei, China. Before she came to the United States, she received a Bachelor degree of Agriculture Structure Environment and Energy Engineering in China Agricultural University. In August of 2011, she joined Dr. Wenqiao Yuan’s group in Kansas State University to start her graduate study in department of Biological and Agricultural Engineering. In January of 2012, she transferred with Dr. Wenqiao Yuan to North Carolina State University to continue her graduate study.
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Chapter 1 Introduction and Literature Review

1.1 Introduction

The microbial fuel cell (MFC) has gained much attention because of its ability to generate power from organic or inorganic compounds via microorganisms. Around one hundred years ago, the technology of generating electricity through bacteria was found (Potter, 1911), but it did not gain much attention. Due to the ability to convert chemical energy to electrical energy, MFCs have many potential applications, such as electricity generation, bio-hydrogen production, wastewater treatment and biosensor (Du et al., 2007). Therefore, the number of studies focused on MFCs increased greatly since early 1990s. Many previous studies concentrated on mediator-MFCs as the power generation could be enhanced. The mediators facilitate the electron shuttling so that the power output can be increased significantly, but the mediators, such as potassium ferricyanide, limit the development of MFCs because of their toxicity to microorganisms and cost (Emde et al., 1989; Bond et al., 2002). Since most of the mediators are expensive and toxic, a microbial fuel cell employing mediator has not been commercialized. In 1999, it was found that mediators did not have to be added to MFCs, which was a significant development in MFCs (Kim et al. 1999). This type of MFCs was a new generation at that time and classified as mediator-less MFC. Many studies on mediator-less MFC focused on discovering the mechanisms of how bacteria transfer electrons. Electron transfer mechanisms will be discussed in the following section of this review. By reducing the expense by eliminating the mediators, MFCs became more attractive in real application, for instance, wastewater treatment and power generation (Ieropoulos et al., 2005).
Microorganisms oxidize substrates in the anodic chamber to produce electrons and protons, while producing carbon dioxide as an oxidation product. Electrons attached on anode (negative terminal) flow to the cathode (positive terminal) through an external circuit. Protons migrate across the proton/cation exchange membrane to combine with electrons to form water if oxygen is provided (Logan et al., 2006a) or to form ferrocyanide if ferricyanide is provided (Ieropoulos et al., 2005). Therefore, a positive current flows from the positive terminal to the negative terminal and this direction is opposite to electron flow. This is how MFCs generate electricity through microorganisms (Fig. 1). In MFCs, oxidation of organic carbon sources does not contribute net carbon dioxide to the atmosphere, and there is no need for extensive pre-processing of the fuel or expensive catalysts (Lovely, 2006). These are the major advantages of MFCs over hydrogen fuel cells, however, the power production by MFCs is currently limited mainly due to either high internal resistance or efficiency of the cathodic reaction, and feasibility of scale-up of MFCs is restricted by the high cost of membranes. With the appropriate optimization of architecture or suitable storage of produced energy, microbial fuel cells are able to power a wide range of widely used devices: for example, store the energy in an external storage device (e.g. capacitor) and dispense that energy intermittently in bursts of high-power when needed (Dewan et al., 2010), power sensors for environmental parameters monitoring at various time intervals rather than continuously (Diamond et al., 2008), provide power for implantable medical devices placed in human large intestine by utilizing intestinal contents (Han et al., 2010), power devices placed on the seafloor or under water environment (Lovely, 2006).
The performance of MFCs can be influenced by several factors. Gil et al., (2003) reported that the factors include the rates of substrate oxidation, electron transfer to the electrode by the microbes, the resistance of the circuit, proton transport to the cathode through the membrane, oxygen supply and reduction in the cathode. In recent years, publications related to microbial fuel cell research have increased rapidly with different favors. Several excellent reviews have come out with different emphasis, such as MFC designs and materials (Logan et al., 2006a; Du et al., 2007; Zhou et al., 2011), discoveries of the capabilities of the microorganisms (Logan and Regan, 2006b; Rabaey and Verstraete, 2005c), performance of different substrates for power generation (Pant et al., 2009). The factors of architecture,
electron transfer mechanisms and substrates will be mainly reviewed in this work here. Furthermore, a few promising applications of MFCs will be discussed.

1.2 Architecture of Microbial Fuel Cells

MFCs are being constructed in a diversity of architectures, and different types of MFCs are usually evaluated by power output, Coulombic efficiency, stability, and longevity. Moreover, in the real application, cost of the materials and feasibility scaling up the architectures also need to be taken into consideration.

1.2.1 Two-chamber MFCs

The conventional design of microbial fuel cells consists of one anode chamber and one cathode chamber, which are connected by a bridge and separated by a proton/cation exchange membrane. This typical two-chamber design of MFCs is frequently operated in batch mode and fed-batch mode. The purposes of proton exchange membrane (PEM), such as Nafion 117, are to separate the liquids in each chamber and allow protons to flow from anode to cathode (Kim et al., 2007). Sometimes, PEM can be replaced by cation exchange membrane (CEM), as it is less expensive and stronger (Rabaey et al., 2005b). Furthermore, the CEM in two-chamber MFCs could be replaced by a salt bridge, which consisted of a tube filled with agar and salt and then capped with porous caps (Min et al., 2005a), but the power output was as low as 2.2 mW/m^2, which was due to the very high internal resistance. Both PEM and CEM help to reduce oxygen diffusion into anodic chamber. Liu and Logan (2004a)
demonstrated that if PEM was removed in a single-chamber MFC, the oxygen diffusion increased, although the internal resistance was reduced.

Cathodes used for MFCs are often either catalyst coated carbon electrodes immersed in water, or they are plain carbon electrodes in a ferricyanide solution. If a catalyst coated carbon electrode is used, the dissolved oxygen is the electron acceptor, and the cathodic reaction is \( O_2 + 4H^+ + 4e^- = 2H_2O \). Platinum is a well-known oxygen reduction catalyst. But platinum is expensive so it needs to be either substituted by cheaper, non-noble metal catalysts (e.g. cobalt) or to be reduced in amount on the electrode (Zhao et al., 2006). Trinh et al. (2009) and Zhao et al. (2006) demonstrated that the cathodic reaction was related to the Pt loading on the electrode and power/current density was directly proportional to catalyst loading on the cathodic electrode. For example, the maximum power density was increased over twice as the Pt loading was increased from 0.5 to 3.0 mg Pt/cm\(^2\) (Trinh et al., 2009). In a ferricyanide solution, plain carbon electrode uses ferricyanide as the electron acceptor and the cathodic reaction is \( \text{Fe(CN)}_6^{3-} + e^- = \text{Fe(CN)}_6^{4-} \). Because ferricyanide in the cathode chamber is reduced to ferrocyanide, the chemical must be replaced after it is depleted. Thus, cathodes with ferricyanide are not economical and environmental friendly although the maximum power could increase by 50-80\% if ferricyanide is used instead of dissolved oxygen (Oh et al., 2004). High power densities involved ferricyanide were obtained by Rabaey et al. (2003; 2004) as well. In Rabaey et al.’s studies, power densities were as high as 3.6 W/m\(^2\) and 4.31 W/m\(^2\) with plain graphite electrodes in two-chamber systems, which used \( \text{K}_3\text{Fe(CN)}_6 \) as electron acceptors.
Instead of ferricyanide, oxygen can be constantly replenished by bubbling the water with air, which makes MFC systems more sustainable. That is the reason that many studies suspend cathodes in liquid with sparged air of the two-chamber MFCs. Oh et al. (2004) obtained power generation of 43 mW/m² with dissolved oxygen into Pt-carbon cathode chamber and stated that power densities in two-chamber MFCs are possible to be increased by improvements of cathode, such as increase concentration of dissolved oxygen. However, aerating air into cathodic chamber causes another disadvantage of a two-chamber MFC besides high internal resistance, because aeration consumes more energy. Unlike aqueous-cathode MFCs, air-cathode MFCs does not require the cathode to be placed in water.

1.2.2 Air-chamber MFCs

As mentioned above, power output of two-chamber MFCs can be improved by increasing the efficiency of the cathode, such as using ferricyanide. But two-chamber MFCs are primarily used in laboratory scale and cannot be adapted for continuous treatment of organic matter due to the demand of oxygenated water. In an alternative architecture without aqueous cathode, cathodic electrode is bonded directly to proton exchange membrane so that air can be directly reduced (Liu and Logan, 2004a; Gottesfeld and Zawodzinski, 1997). This is the air-cathode MFC (Liu et al., 2005a) (Fig. 2). The earliest air-cathode MFC architecture was designed by Sell et al. (1989), and they reported that an oxygen gas diffusion electrode could be used as a cathode in bioelectro-chemical fuel cell (Sell et al., 1989). But this air-cathode design has not drawn much attention in MFC research until Liu et al. (2004b) reported the air-cathode MFC could produce much greater power than typical aqueous-cathode ones. In
this study, they developed the air-cathode configuration with presence and absence of PEM, and maximum power output was 262 mW/m² using glucose with PEM and 494 mW/m² without PEM. However, the Coulombic efficiency was much lower with absence of PEM due to oxygen diffusion into the anode. Further tests in Liu et al.’s study (2004b) were conducted with acetate and butyrate in the same reactor in the absence of the CEM. Other studies have been conducted to examine power outputs in air-cathode MFCs, and the results were 506 mW/m² with acetate and 305 mW/m² butyrate in the same air-cathode design without any membrane (Liu et al., 2005a).

Figure 1.2 Schematic diagram of single-chamber microbial fuel cells (Pant et al., 2009).

The architecture of air-cathode MFCs is aimed to optimize some characteristics of two-chamber MFCs, such as low relative power output, high cost of cathode catalysts and membranes, energy requirement for intensive air/oxygen sparging. Another advantage of the
air-cathode over the two-chamber is the reduction of the high internal resistance of MFCs, which is a key factor to enhance electricity production. For example, internal resistance ranged from 1239Ω to 1344Ω among different membranes in aqueous-cathode MFCs and 84Ω-98Ω in air-cathode MFCs (Liu and Logan, 2004a). However, oxygen diffusion to anaerobic anode chamber increases due to cathode exposure to air and removal of proton/anion exchange membrane, which could cause low electron and energy recoveries (Liu et al., 2005a).

Table 1.1 Comparisons of two-chambered and single-chambered MFCs (Lee et al., 2010).

<table>
<thead>
<tr>
<th>Two-chamber MFCs</th>
<th>Single-chamber MFCs</th>
</tr>
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<tbody>
<tr>
<td>Advantages</td>
<td></td>
</tr>
<tr>
<td>• Well-controlled conditions;</td>
<td>• Increase mass transfer to cathode;</td>
</tr>
<tr>
<td>• Essentially architecture for</td>
<td>• Decrease operating cost;</td>
</tr>
<tr>
<td>testing concepts, e.g., bacterial</td>
<td>• Decrease overall reactor volume;</td>
</tr>
<tr>
<td>activities, optimizing materials;</td>
<td></td>
</tr>
<tr>
<td>Disadvantages</td>
<td></td>
</tr>
<tr>
<td>• High internal resistance;</td>
<td>• Loss of anaerobic condition in anode chamber.</td>
</tr>
<tr>
<td>• High cost due to PEM/AEM and</td>
<td></td>
</tr>
<tr>
<td>cathodic catalyst/electrolyte;</td>
<td></td>
</tr>
</tbody>
</table>

1.2.3 Other designs of MFCs

Voltage generated by MFCs remains limited and cannot exceed a theoretical open circuit voltage of 1.14 V as determined by the NADH (-0.32 V) and pure oxygen (+0.82 V) redox
potentials, even neglecting the internal losses (Madigan et al., 2000; Aelterman et al., 2006). Other optimized architectures of MFCs were constructed. For example, tubular/up flow architecture (Jang et al., 2004; He et al., 2005; He et al., 2006) and stacked microbial fuel cells (Aelterman et al., 2006).

Jang et al. (2004) designed a tubular reactor architecture working in continuous flow mode, the flow moving through an anode chamber and then directly up into the cathode chamber in the same column. They claimed the up-flow reactor had several advantages over conventional designs, such as a higher affinity for oxygen with cathode (Jang et al., 2004), combining the benefits of the up-flow anaerobic sludge blanket system with two-chamber MFC (He et al., 2005). These advantages result in improving both electricity generation and wastewater treatment.

Referring to the idea of connecting several fuel cells in series to add the voltages, connecting several MFCs in series or parallel can enhance voltage or current output. Aelterman et al. (2006) designed a stacked system of MFCs, which was used to examine the performance of MFCs connected in series or parallel. In this system, the separated MFCs were electrically connected in series, or parallel using copper wires connected to the electrodes and held together by screw bolts. The result obtained by Aelterman et al. (2006) demonstrated that the parallel-connected system could exhibit higher maximum bioelectrochemical reaction rate. Applying stacked MFCs to wastewater treatment application can enhance the chemical oxygen demand removal compared to a single cell.
All of the optimizations of MFCs’ configuration or architecture aim to reduce the internal resistance and increase the cell power output. Chae et al. (2009) claimed better understanding of the bacterial community or dominant species, which contributed to the exoelectron transfers, could help to achieve better performance of MFCs.

1.3 Microorganisms Inoculated in Microbial Fuel Cells

Fuel cells are able to generate electricity from many different chemicals by oxidation of the chemicals at the anode and reduction at the cathode. MFCs do not need to use metal catalysts at the anode, instead, they use microorganisms that biologically oxidize organic matter and transfer electrons to the electrode. Logan (2009) defined the microorganisms as exoelectrogens due to their capability of exocellular electron transfer. Other researchers described the microorganisms as electrochemically active bacteria (Chang, et al., 2007), anode respiring bacteria (Rittmann, et al., 2008) and electricigens (Lovley, 2006).

The microorganisms, which can be inoculated in MFCs for electricity generation, are found in marine sediment, soil, wastewater, fresh water sediment or activated sludge (Niessen et al., 2006). A number of species, such as Geobacter, Shewanella, Pseudomonas, Clostridium and Desulfuromonas, are often inoculated into MFCs or MECs for electricity and hydrogen productions, and they are able to oxidize acetate, ethanol, lactate, butyrate, or propionate as substrate (Pant et al., 2009). Therefore, the electron transfer mechanisms in anode chamber of MFCs are a crucial issue of studying MFCs’ working principles. So far, there are several known mechanisms of how bacteria transfer electrons to electrode surfaces (Fig. 3).
Figure 1.3 Schematic diagrams of electron transport in microbial fuel cells (Oh et al, 2010; Logan, 2009).

Note: outer membrane cytochrome or extracellular substance; bacterial pili network; electron shuttling and diffusion.

In MFC, the anodic electrode potential is developed when electrons are available to the electrode. But some bacterial species cannot transfer or release electrons to electrode through their electron transport systems because of the non-conductive nature of the cell surface structures (Jang, et al., 2004; Ieropoulos, et al., 2005). Thus, electrochemical mediators are introduced to assist electron transfer from the microbial cells to the electrode. Mediators penetrate the bacterium cell in their oxidized form and interact with reducing agents within the cell. After being reduced, the mediators are also cell permeable and are capable of
diffusing out of the cells to attach to the electrode surface. Then, the reduced mediators are electrocatalytically oxidized by transfer electrons. The oxidized mediators are free to start over this cycle again (Ieropoulos, et al., 2005). However, the mediators are usually toxic phenolic compounds. Therefore, the long-term operation of mediated MFCs cannot be achieved and mediated MFCs have limited commercial applications (Kim et al., 2003).

Besides of the bacterial species assisted by mediators to transfer electrons above, some bacteria are able to transfer the electrons oxidized from organic matters to electrodes without the mediator. MFCs involved in bacteria, which do not need any mediator to transfer electrons, are classified as mediator-less MFCs. In most of the mediator-less MFCs, the anodes are often inoculated with dissimilar metal reducing microorganisms, including the species of Shewanella, Rhodoferax and Geobacter. The performance of MFCs is impacted not only by types of microorganisms presented, but also by mechanisms of electrons transfer to anode. Several mechanisms are involved in mediator-less MFCs: bacteria can transfer electrons through self-produced mediators; electrons transfer is related to nanowires produced by bacteria; in an absence of nanowires, electrons can be transferred via the surface of bacterial cells as well (Lower et al., 2001). For the self-produced mediators to transfer electrons, Rabaey et al. (2005a) demonstrated that Pseudomonas aeruginosa could produce electron shuttles to enhance electron-transfer rate. Geobactor and Shewanella species are capable of producing nanowires, which contribute to transfer electrons (Gorby and Beveridge, 2005; Reguera et al., 2005).
*Geobacter sulfurreducens* is a strict anaerobic chemoorganotroph which oxidizes acetate with Fe(III), S, Co(III), fumarate, or malate as the electron acceptor and contains c-type cytochromes (Caccavo et al., 1994). Bond and Lovely (2003) stated that *Geobacter sulfurreducens* could oxidize organic substrates completely to transfer electrons to electrodes without mediators. After this statement, Gorby and Beveridge (2005) concluded that nanowires were produced by *Geobacter sulfurreducens* in response to electron acceptor limitation, which resulted in the high efficiency of electrons transfer. *Shewanella* species is a gram-negative, dissimilatory metal-reducing bacterium found in soils, sediments, surface waters, and ground waters. They are widely studied in MFCs research area due to their ability to conserve energy for growth by using oxygen or ferric iron as a terminal electron acceptor.

The electron transferring mechanisms of *Geobacter* and *Shewanella* species are well studied because they exhibit promising capabilities of electricity producing and hydrogen generating in MFCs and MECs systems, respectively. *Shewanella oneidensis* MR-1 could produce electrically conductive nanowires by responding to electron acceptors’ limitation (Gorby et al., 2006). For electricity generation, *Geobacter sulfurreducens* could produce electricity of 2.15kW/m$^3$ with acetate; the highest MFCs power density reported to date (Nevin et al., 2008). Besides, the production of hydrogen by *Geobacter sulfurreducens* was approximately 40Pa (hydrogen partial pressure) after electron acceptor-limited growth with 20mM acetate and 20mM fumarate (Cord-Ruwisch et al., 1988).
Other than using pure culture in anode of MFCs, mixed cultured microorganisms have good performances, as well. Rabaey et al. (2003) reported that mixed cultures showed higher performance than isolated pure cultures in MFCs with benefit of much wider substrate utilization. There are both electrophiles/anodophiles in mixed cultures, so mixed microorganisms use natural mediators together in the same chamber. Moreover, Oh et al. (2010) claimed the mixed populations would be more robust for environmental changes, for example, temperature changes, substrates loading rate changes. The mixed populations could shift metabolic pathways to make adaptation to the new environment.

1.4 Substrates Oxidized by Microbes

Many studies emphasize on exploring performance of MFCs with different substrate. The substrate is a significant factor in any biological process because it serves as carbon (nutrient) and energy source. In MFCs, the bacterial abilities to oxidize substrates and transfer electrons are directly related to production of current (Pant et al., 2009). Also, Chae et al. (2009) stated the substrates influence not only the integral composition of the bacterial community in the anode biofilm, but also the MFCs performance, such as power density and Coulombic efficiency (CE). Besides, Liu et al. (2009) regarded substrate as one of the most important biological factors affecting electricity generation in MFCs.

Many categories substrates can be fed into MFCs, such as non-fermentable substrates (acetate, butyrate), fermentable substrates (glucose, xylose, sucrose), complex substrates (domestic wastewaters, food process wastewaters, paper recycled wastewaters) (Catal et al.,
For example, Chae et al., study (2009) compared the performance of four different substrates in terms of CE and power density in two-chambered MFCs, which were inoculated anaerobic digester sludge. In this study, the tested substrates were acetate, butyrate, propionate and glucose, and acetate fed-MFC showed the highest CE (72.3%), following by butyrate (43.0%), propionate (36.0%) and glucose (15.0%).

Although many sorts of substrates could be oxidized by different species of bacteria, Pant et al. (2009) declared that it was difficult to make comparisons of MFCs performance with different substrates. It is mainly due to researchers using different operating conditions (e.g. surface area and types of electrodes), different inoculated microorganisms, different designs and volume of reactors. Table 2 shows some comparisons of power outputs with different substrates under different operational conditions.
Table 1.2 Power output with different substrates under different operational conditions.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Concentration</th>
<th>Power output</th>
<th>MFC design</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>800 mg/L</td>
<td>506 mW/m²</td>
<td>Single-chamber</td>
<td>Liu et al. (2005)</td>
</tr>
<tr>
<td>Glucose</td>
<td>500-3000 g/L</td>
<td>3600 mW/m²</td>
<td>Two-chamber</td>
<td>Rabaey et al. (2003)</td>
</tr>
<tr>
<td>Butyrate</td>
<td>1000 mg/L</td>
<td>305 mW/m²</td>
<td>Single-chamber</td>
<td>Liu et al. (2005)</td>
</tr>
<tr>
<td>Domestic wastewater</td>
<td>210-220 mg/L</td>
<td>26 mW/m²</td>
<td>Single-chamber</td>
<td>Liu et al. (2004b)</td>
</tr>
<tr>
<td>Swine wastewater</td>
<td>COD</td>
<td>261 mW/m²</td>
<td>Single-chamber</td>
<td>Min et al. (2005b)</td>
</tr>
<tr>
<td>Artificial wastewater</td>
<td>mg/L COD</td>
<td>170 mW/m²</td>
<td>Up-flow MFC</td>
<td>He et al. (2005)</td>
</tr>
<tr>
<td>Carboxymethyl cellulose</td>
<td>1000mg/L</td>
<td>143 mW/m²</td>
<td>Two-chamber</td>
<td>Ren et al., (2007)</td>
</tr>
<tr>
<td>Corn stover</td>
<td>N/A</td>
<td>331 mW/m²</td>
<td>Single-chamber</td>
<td>Wang et al. (2009)</td>
</tr>
</tbody>
</table>

Note: The power density normalized by surface area is calculated by the formula of $P = \frac{E_{MFC}}{A \times R}$, where $E_{MFC}$ is measured voltage across the load, $A$ is surface area of both sides of anodic electrode, $R$ is the load.
1.4.1 Acetate

Acetate is the end product of several metabolic pathways for higher order carbon sources and has been chosen as substrate for MFCs in plenty of researches, especially for evaluating the performance of new MFCs components, reactor designs or operational conditions. Bond et al. (2002) claimed that acetate could be a carbon source to induce electroactive bacteria, which led acetate to be used in MFCs studies extensively. And Aelterman (2009) claimed the common use of acetate was because of its inertness towards alternative microbial conversions at room temperature, such as fermentations or methanogenesis. Acetate is the most preferred substrate for electricity generation with higher power density and CE (Chae et al., 2009; Liu et al., 2005a; Aelterman et al., 2006; Jung and Regan, 2007).

1.4.2 Glucose

Glucose is another commonly used substrate in MFCs. By some comparisons of glucose with other substrates in the same operation conditions, MFCs with glucose exhibit higher power output than anaerobic sludge (Hu, 2008), lower energy conversion efficiency than acetate (Lee et al., 2008) and lower Columbic efficiency (Chae et al., 2009). And Chae et al. (2009) declared that the lower Columbic efficiency was because of glucose might be consumed by bacteria, which could not produce electricity. This emphasizes the advantage of acetate used as substrate in MFCs.
1.4.3 Lignocellulosic biomass

Lignocellulosic materials from agricultural residues are abundant, renewable and cost-effective feedstock for energy production. However, they cannot be directly oxidized by microorganisms in MFCs, so some pretreatment is needed to convert lignocellulosic materials into monosaccharides (Ren et al., 2007). Electricity generation from cellulose in a two-chamber MFC using a coculture of bacteria was performed by Ren et al. (2007). The maximum power density was 143 mW/m$^2$ with ferricyanide as the catholyte. Wang et al. (2009) examined the performance of a single-chamber MFC with mixed culture from corn stover, and obtained a maximum power density of 331 mW/m$^2$.

1.4.4 Other designs of MFCs

Liu et al. (2004b) demonstrated MFCs could produce electricity from domestic wastewater with removal of chemical oxygen demand. Many types of wastewater can be used as substrates in MFCs, because the production of intermediates in wastewater helps electricity generation. Min et al. (2005b) demonstrated that power densities generated from swine wastewater were 45 mW/m$^2$ with two-chamber MFC and 261 mW/m$^2$ with single-chamber MFC, respectively. He et al. (2005) reported that up-flow MFC generated electricity with a maximum power density of 170 mW/m$^2$ from artificial wastewater.

1.5 Applications of Microbial Fuel Cells

Along with the understanding of the MFC concept, many MFC-based applications have emerged, such as wastewater treatment, microbial electrolysis cells, sediment MFCs and
bioremediation. Several of MFCs applications will be explained in this section. Among those MFC-based technologies, the most immediate and useful one is as a method of wastewater treatment (Logan and Regan, 2006c). The electricity produced by MFCs can be used for powering other technologies, such as biologically inspired robots, some small devices, or remote devices. In addition, the voltage generated by MFCs can be used on microbial electrolysis cells (MECs), which is a modified MFC-based system to produce $\text{H}_2/\text{H}_2\text{O}_2$ instead of electricity. However, most of the MFCs applications are limited to lab-scale systems because of some practical difficulties, such as economic or environmental feasibilities.

In the aspect of environmental feasibility, using buffer solution to keep the pH balanced in MFC plants is not practical for large-scale. Harnisch and Schröder (2009) stated that adding buffer salts (e.g. phosphate or carbonate) would result in an increase of CO$_2$ emission, even the idea of using such chemicals in practice would be noneconomic. Instead of buffer salts, using CO$_2$ or bicarbonate to buffer the pH shift is a better option (Pant et al., 2011). In the aspect of economic feasibility, the high cost of current MFC-base technologies is contributed to the electrode material cost and membrane cost. The high cost of electrode material is mainly due to the use of platinum, which is used as a catalyst. In recent years, some researchers focus on alternative catalyst to replace platinum. Some cost-effective catalysts have been examined for MFCs, such as CoTMPP, iron phthalocyanine, manganese dioxide, activated carbon or nickel powder (Pant et al., 2011). Therefore, some modifications of the
basic MFC systems or alternative component materials can help to overcome the difficulties of scaling-up MFC-based applications or technologies.

1.5.1 Bio-hydrogen production

Due to the energy intensive and environmental friendly issues, biological hydrogen (bio-hydrogen) production processes are found to be advantageous over thermochemical and electrochemical processes. Dark fermentation can produce bio-hydrogen, but the efficiency is low. For example, fermentation of carbohydrate-rich wastewater was generally less than 15% (Angenent et al., 2004). Besides, methanogenic consumption of hydrogen in the fermentation process resulted in the majority of substrates being converted to acetate or butyrate as byproducts (Rozendal et al., 2006). However, conversion of these byproducts involves endothermic reactions so that these byproducts cannot be further converted to hydrogen without an external energy input (Logan et al., 2008). Alternatively, MFCs can be modified to produce hydrogen instead of electricity by adding a small amount of electricity (0.11V theoretically) and removal of oxygen at cathode (Fig. 4). By combining bacterial metabolism with electrochemistry, bio-hydrogen can be produced by the modified MFC systems, which is called microbial electrolysis cells (MECs) (Liu et al., 2005b). MECs’ efficiency is relative to the power input (>0.2 V typically), but it is much less than the electrical energy needed for water electrolysis at neutral pH (1.21 V theoretically, >1.6-1.8 V practically) (Call and Logan, 2008). For bio-hydrogen production, MEC systems are not only advantageous over electrolysis of water, but also over conventional fermentation: MECs can produce 8-9 mol H₂/mol of glucose while fermentation processes produce 4 mol H₂/mol of glucose with
acetate produced (C₆H₁₂O₆ + 2H₂O → 4H₂ + 2CO₂ + 2C₂H₄O₂). While oxygen diffusion into anode chamber in an MFC reduces electron recovery (Coulombic efficiency), MECs should have greater electron recoveries with oxygen removal from the cathode chamber.

The very first MEC system was designed by Liu et al. (2005b), which was only for “proof of concept” and was not optimized. This reactor was a simple two-chambered reactor consisting of two glass bottles separated by a CEM, and the gas was release from the headspace in cathode chamber and then collected. Later, some architectures of MECs were optimized by several ways, for example, increasing the size of the membrane relative to the electrode-projected surface area, using anodic electrode with larger surface area (e.g. graphite granules),

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Figure 1.4 Operational principles of microbial electrolysis cells (Liu et al., 2006b).
reducing electrode spacing (Cheng and Logan, 2007; Rozendal et al., 2007). Cheng and Logan (2007) developed a compact MEC system, which used chemically modified three-dimensional graphite granule as electrode and an anion exchange membrane. The H₂ yield of this study was ranged from 3.03 to 3.95 mol/mol of acetic acid when the applied voltage was from 0.3 to 0.8 V.

As mentioned above, the external power source is needed to provide the energy input, which is required to drive the hydrogen production reactions in the microbial electrolysis process. In laboratory tests, two different devices are used to provide the voltage: a power supply unit or a potentiostat. Although the voltage supply in MECs is lower than that for water electrolysis, it still consumes huge amount of energy if scaling-up the reactors (Manish and Banerjee, 2008). Thus, reducing voltage supply or providing the voltage by sustainable electricity generation processes is one of the key issues in developing the efficient and cost-effective MECs. Since the open circuit voltage of an MFC can reach as high as 0.80 V (Liu et al., 2005a), the voltage needed for an MEC can be supplied by an MFC. Enlightened by this idea, Sun et al. (2008; 2009) developed a MEC-MFC-coupled system for bio-hydrogen production, which means the external electric power supply for an MEC is provided by an MFC. This system is an effective way to use the power generated from MFCs. The yield of H₂ in the MEC was 1.60±0.08 mol/mol of acetate with 100 mM of phosphate buffer in the MFC. Moreover, Sun et al. (2009) examined that hydrogen production could be significantly enhanced if several MFCs connected in series to supply power for the MEC.
Table 1.3 Comparisons of the H$_2$ production performance of MECs with different substrates and power supplies.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>MEC design</th>
<th>Cathode catalyst</th>
<th>$H_2$ production rate (m$^3$m$^{-3}$day$^{-1}$)</th>
<th>Power supply (Volt)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>Two-chamber</td>
<td>0.5 mg Pt/cm$^2$</td>
<td>0.37</td>
<td>0.45</td>
<td>Liu et al. (2005)</td>
</tr>
<tr>
<td>Glucose</td>
<td>Two-chamber</td>
<td>0.5 mg Pt/cm$^2$</td>
<td>1.23</td>
<td>0.6</td>
<td>Cheng and Logan (2007)</td>
</tr>
<tr>
<td>Butyrate</td>
<td>Two-chamber</td>
<td>0.5 mg Pt/cm$^2$</td>
<td>0.45</td>
<td>0.6</td>
<td>Cheng and Logan (2007)</td>
</tr>
<tr>
<td>Wastewater</td>
<td>Two-chamber</td>
<td>0.5 mg Pt/cm$^2$</td>
<td>0.01</td>
<td>0.5</td>
<td>Ditzig et al. (2007)</td>
</tr>
<tr>
<td>Swine waste</td>
<td>Single-chamber</td>
<td>0.5 mg Pt/cm$^2$</td>
<td>1</td>
<td>0.5</td>
<td>Wanger et al. (2009)</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Single-chamber</td>
<td>10 wt.% Pt</td>
<td>2</td>
<td>0.9</td>
<td>Selembo et al. (2009)</td>
</tr>
<tr>
<td>Cellulose</td>
<td>Two-chamber</td>
<td>0.5 mg Pt/cm$^2$</td>
<td>0.11</td>
<td>0.6</td>
<td>Cheng and Logan (2007)</td>
</tr>
</tbody>
</table>
Note: Often used approaches to collect produced gas: glass syringes and gas bags. As hydrogen is a small molecule that easily permeates through tubing and connections, it is very important that the reactor design is gastight with proper seals. The use of large lengths of tubing and fittings for continuous flow devices should be avoided to reduce hydrogen gas losses, especially when gas production rates are low (Logan et al., 2008).

Hydrogen can be successfully produced from cellulose, glucose, acetate, butyrate, and wastewater in MECs (Table 3). The hydrogen production rate from cellulose in an MEC was low compared to single volatile fatty acid (Cheng and Logan, 2007). In order to optimize the conversion of lignocellulosic biomass into hydrogen, Lalaurette et al. (2009) developed a two-stage process combining dark-fermentation and electrohydrogenesis for hydrogen production from recalcitrant lignocellulosic materials, and the overall hydrogen yield was 9.95 mol of H₂/mol of glucose. This result indicated that a higher gas production rate could be achieved using a two-stage fermentation and MEC process.

1.5.2 Wastewater treatment

So far, the most successful and widely used biological technology for wastewater treatment is the activated sludge process. In the process, pumping and aeration are the predominant energy consuming, for example, 21% of the total treatment energy demand consumes by pumping and 30-55% consumes by aeration (Oh et al., 2010). Due to the high cost of operation and huge demand of energy, alternative approaches to treat wastewater are favored.
The working principle, as mentioned in the introduction section above, exhibits the ability of MFCs to treat wastewater with benefits of low energy requirement and additional energy production.

The first demonstration of MFCs using domestic wastewater as the substrate was reported by Liu et al. (2004b). They used single-chamber MFCs, which did not need any oxygen aeration into the cathode chamber, and the COD removing rate of domestic wastewater was up to 80%. Then Rabaey et al. (2005b) demonstrated up to 96% of the organic matter in wastewater was converted to electricity by a tubular, single-chamber MFC. Min et al. (2005) demonstrated soluble COD removal efficiency was over 90% in an up-flow MFC using artificial wastewater as substrate.

1.5.3 Biosensor

Besides the applications of MFCs mentioned above, another potential application of the MFC technology is to use it as a sensor for pollutant analysis (Logan et al., 2006a). Kim et al. (2003) found that there was a proportional correlation between the Coulombic yield of MFCs and the strength of the wastewater. Therefore, MFCs without mediators using electrochemically-active, metal-reducing bacteria are a good approach to examine biological oxygen demand (BOD) in wastewater. Furthermore, Moon et al. (2004) did some efforts to optimize the response time and sensitivity of MFCs used as continuous BOD sensor. There are some advantages of using MFCs as BOD sensor, such as microorganism variety, operational stability and high accuracy (Kim et al., 2003).
1.5.4 Sediment MFCs

Another exciting application in microbial fuel cell research is the development of an MFC that can harvest electricity from the organic matter in aquatic sediments (Reimers et al., 2001; Tender et al., 2002). The purpose of sediment MFC design is to power devices placed on the seafloor or under water environment, where it will be expensive and technically difficult to exchange traditional batteries routinely, and the sediment MFC is also known as benthic unattended generators (BUGs) (Lovely, 2006). The idea of sediment MFC design is to place the anode into the anaerobic sediment and place the cathode into the overlying water containing dissolved oxygen. As the exoelectrogenic bacteria are rich in the sediments, the sediment MFCs are ready to produce electricity. Reimers et al. (2001) proposed that the sediment-anode combined with seawater-cathode configuration harvested energy from the net oxidation of marine sediment organic matter.

1.6 Life Cycle Assessment of MFCs

As microbial fuel cells and microbial electrolysis cells are recently developed technologies, the environmental costs and benefits of MFCs and MECs have to be verified. This can be done by life cycle assessment (LCA), which is a technique to access the potential environmental impacts caused by a process or a product. The results of LCA reveal the true potential and identify the environmental impacts associated with the evaluated product or process, for example, energy and materials usage, waste discharges, impacts of these wastes on the environment (Lam et al., 2009; Pant et al., 2011).
Foley et al. (2010) conducted a LCA to compare the environmental impacts of three technologies of wastewater treatments: anaerobic treatment (biogas generation), a microbial fuel cell (direct electricity generation) and a microbial electrolysis cell (hydrogen peroxide production). In this study, the major conclusion was that a microbial electrolysis cell provided more significant environmental benefits compared to anaerobic wastewater treatment, however, a microbial fuel cell did not. More specifically, the MEC had more significant net positive impacts than both anaerobic digestion and the MFC, and anaerobic digestion treatment had more significant net positive impacts than the MFC. Although the positive benefits in the MFC (electricity generation) were large enough, there were two underlying drawbacks: the attendant uncertainty in the data and the calculation, and the contingent result on optimistic design assumptions. In addition, they concluded that the MEC had highly positive benefits due to it could directly produce pure H₂/H₂O₂ without greenhouse gases emission.

1.7 Summary

Microbial fuel cells (MFCs) are new types of bioreactors that use exoelectrogenic biofilms for electrochemical energy production. In recent years, a large number of studies have been conducted to explore microbial fuel cells in many aspects, such as electron transfer mechanisms, enhancing power outputs, reactor developments and applications. Although MFCs are a promising technology for renewable energy production, they face several challenges, as well. For instance, they possess low levels of power density, scale-up feasibility, high cost of component materials, and large internal resistance. In the author’s
opinion, combinations of MFCs or MECs with other high value byproducts generating processes have a bright future in sustainable energy research.
REFERENCES


Chapter 2 Thesis Objectives

Firstly, to better understand the effects of operational parameters on the performance of microbial fuel cells (MFCs) for electricity generation and H₂ production, specific goals and approaches were as follows:

1. To explore the effect of different substrate concentrations (10mM ~ 80mM) on growth of *G. sulfurreducens* and electricity generation by two-chambered MFCs with two membranes (CEM and PEM).

2. To examine the ability of two-chambered MFCs for electricity generation as a function of electrode surface areas (14 cm² ~ 42 cm²) or medium conductivity (9.12 mS/cm ~ 28.0 mS/cm).

3. To investigate the effect of applied voltage (0.3 V ~ 1.05 V) on hydrogen production by two-chamber MECs.

Secondly, a two-stage process by combining dark-fermentation with MFCs/MECs was developed for electricity generation and H₂ production from hemicellulosic materials. It was designed to investigate the performance of MFCs/MECs by using the acetic acid from anaerobic fermentation as feedstock. Specific goals and approaches were as follows:
4. To explore the acetic acid production by *M. thermoacetica* under different temperatures (30 °C and 60 °C) and with different initial xylose loadings (10 g/L, 20 g/L and 30 g/L).

5. To use corncob hydrolysate, which was pretreated by dilute 1% H₂SO₄ with 10% (w/w) solid loading at 121 °C for 30 minutes, as the fermentation substrate for acetic acid production by *M. thermoacetica*.

6. To feed the acetic acid, which was fermented from xylose medium or corncob hydrolysate, into anode chamber of MFCs/MECs instead of sodium acetate. Both pure culture and mixed culture of *G. sulfurreducens* and *M. thermoacetica* were explored for electricity generation and H₂ production.
Chapter 3 Optimization of Electricity and H₂ Generation
by *Geobacter sulfurreducens*

**Abstract:** The objective of this chapter was to understand and optimize the performance of MFCs for electricity generation and MECs for hydrogen production by varying substrate concentration, electrode surface area, medium conductivity and applied voltage. Two membranes were configured into MFCs/MECs, which were cation exchange membrane (CEM) and proton exchange membrane (PEM). The power density, Coulombic efficiency and H₂ yield were examined. The MFCs operated with CEM had better performance for electricity generation than PEM with other operational parameters fixed. While, PEMs outperformed over CEMs for hydrogen gas production by the MECs. The electricity generation by MFCs was positively related to both substrate concentration and electrode surface area. It meant that, in an MFC with other operational parameters fixed, a higher power density resulted from a higher substrate loading or a larger electrode surface area, and vice versa. For the hydrogen gas production by the MECs, an increase of applied voltage resulted in an increase of H₂ yield. In detail, for electricity generation by MFCs with CEMs, the MFC loaded with a sodium acetate concentration of 20 mM, an electrode surface area of 42 cm² and 0.1 g/L of KCl in anodic solution showed the highest power density (79.21 mW/m²) and highest Coulombic efficiency (15.5%) among all treatments. For hydrogen production by MECs with PEMs, the MEC driven by a sodium acetate concentration of 20
mM, an electrode surface area of 42 cm² and applied voltage of 0.954 V resulted in the highest H₂ yield (160.56 mM of H₂/mol of acetate) among all treatments.

3.1 Introduction

The microbial fuel cell (MFC) has gained much attention as its ability to generate power from organic or inorganic compounds via microorganisms. Based upon the ability of converting chemical energy to electrical energy, MFCs have many potential applications, such as electricity generation, bio-hydrogen production, wastewater treatment and biosensor (Du et al., 2007). MFCs have been found to be advantageous over hydrogen fuel cells on some aspect. Firstly, oxidation of organic carbon sources does not contribute net carbon dioxide to the atmosphere. Additionally, there is no need for extensive pre-processing of the fuel or expensive catalysts (Lovely, 2006). Furthermore, the widely renewable sources can be used as substrates (Fan et al., 2008).

In MFC system, substrates can be oxidized to produce electrons and protons by microorganisms in the anodic chamber. Protons migrate across the membrane to combine with electrons to be reduced, forming water if oxygen is provided (Logan et al., 2006) or reducing ions (e.g. forming ferrocyanide if ferricyanide is provided) (Ieropoulos et al., 2005). Therefore, a positive current flows from the cathode to anode, which is opposite to the direction of electron flow. For example, if operates MFCs with acetate as the substrate, which is the electron donor, the reaction is (Logan et al., 2006):

\[
CH_3COO^- + 3H_2O \rightarrow 2CO_2 + HCO_3^- + 8H^+ + 8e^- \quad (E' = -0.284V) \quad (1)
\]
where $E'$ is the anode potential adjusted for pH=7.0 at 298 K.

In anode chamber, the electrode acts as the electron acceptor, leading the electrons flow from the anode to cathode chamber through an external load (e.g. resistors or devices). If oxygen is used as electron acceptor, the reaction is (Logan et al., 2006):

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (E' = 0.805V) \quad (2)$$

where $E'$ is the cathode potential adjusted for pH=7.0 at 298 K. Therefore, if an MFC is operated with acetate oxidizing as substrate in anode and oxygen reducing in cathode at pH=7, the overall cell electromotive force (emf) is equal to $0.805V - (-0.284V) = 1.089V$. The emf is defined as the potential difference between cathode and anode, and it can be understood as the maximum potential of the system.

If there is no electron acceptor presented in the cathode, electrons are forced to combine with protons to produce hydrogen gas by adding the external power supply, which is to overcome the thermodynamical barrier (Liu et al., 2005b):

$$2H^+ + 2^- \rightarrow 2H_2 \quad (E' = -0.414V) \quad (3)$$

where $E'$ is the cathode potential adjusted for pH=7.0 at 298 K. Therefore, hydrogen can be produced at the cathode chamber by applying a voltage at least $-0.284V - (-0.414V) = 0.130V$.

However, the actual cell voltage of an MFC is lower than the emf, which is a theoretically maximum attainable voltage. Therefore, the power production is currently limited due to
several factors, such as ohmic loss, activation loss, bacterial metabolic loss, and concentration loss (Logan et al., 2006). As the measured cell voltage ($E_{\text{cell}}$) is linear to current ($I$) based on Ohm’s law, it can be described as

$$E_{\text{cell}} = OCV - IR_{\text{int}} \quad (4)$$

where $OCV$ is open circuit voltage, and $R_{\text{int}}$ is internal resistance. Thus, the measured cell voltage can be enhanced by a reduction of the internal resistance, and it is important to quantify and investigate the internal resistance of MFCs. The internal resistance is composed of anode resistance, cathode resistance, electrolyte resistance and membrane resistance (Fan et al., 2008).

Many studies have been conducted to understand how the internal resistance contributes or to compare the internal resistance in different MFC configurations and materials. Liang et al. (2007) stated that, in MFCs, the composition of internal resistance includes anodic resistance, cathodic resistance, and ohmic resistance. And the ohmic resistance results from proton exchange membrane (PEM) and electrolyte. Fan et al. (2008) conducted a further investigation of the contribution of each component of single-chamber membrane-free MFCs. The result indicated the anode contributed 5.4% of the internal resistance, and the cathode and electrolyte contributed of the rest with equal weight (47.3% of each). Oh and Logan (2006) explored the performance of two-chamber MFCs based on electrode surface area and PEM surface area. Besides, Ghangrekar and Shinde (2007) obtained the results that less anode surface area (70.21 to 210.64 cm$^2$) could achieve higher power output in membrane-less two-compartment MFCs.
As mentioned above, the power is limited by several types of losses (Logan et al., 2006), it is mainly due to resistance to the flow of electrons through electrodes and that of ions through membranes, but also occur in electrons transferring process from or to a compound at the electrode surface. The main function of the membrane in two-chambered MFCs is to separate the liquid and allow protons to pass between the two chambers. As Kim et al. (2007) stated, another function of the membrane is to reduce oxygen diffusion from cathode to anode chamber, which helps to develop the anaerobic condition for bacteria growth, especially pure strains. PEM (e.g. Nafion 117) is used in most chemical fuel cells and many MFC systems, while CEM (e.g. CMI-7000) is used in MFC systems as well because CEM costs less and is stronger than Nafion 117 (Kim et al., 2007). For Nafion membranes, the excellent proton conductivity makes it widely used in MFC systems, however, it has been involved several operational problems. Rozental et al. (2006) stated Nafion membrane could also transport the cation ions other than proton, such as Na⁺, K⁺, NH₄⁺, because those ions concentration could be 10⁵ times higher than proton produced by the microbes.

Besides electricity generation, bio-hydrogen production is another promising application of the MFCs. Hydrogen has gained much attention recently because it is recognized as an ideal renewable energy source without producing greenhouse gas. If its producing cost can be reduced, hydrogen has been considered as one of the most promising future fuel (Dunn, 2002). Biological processes for hydrogen production are significantly promising because they can achieve bioremediation besides H₂ production. Several processes have been well studied: biophotolysis (direct or indirect), photofermentation, dark fermentation etc.
(Hallenbeck and Benemann, 2002). Microbial electrolysis cell (MEC), also referred as bioelectrochemically assisted microbial reactor, is an emerging technology that utilizes the electrochemical hydrogenation for the direct transformation of biologically degradable material into hydrogen (Ditzig et al., 2007). MEC is modified based on microbial fuel cell (MFC) to produce hydrogen with high efficiency, which combines bacterial metabolism with electrochemistry. The bacteria oxidize simpler organic compounds in anode, and H₂ is generated at cathode in MECs (Liu et al., 2005b). For bio-hydrogen production, MEC systems are advantageous over conventional fermentation, because MECs can achieve high H₂ yield (H₂-capature efficiency 67%~91%) and utilize end products of fermentation (e.g. acetate or butyrate) (Lee et al., 2010).

As mentioned above, external power source is needed to provide the energy input required for driving the hydrogen production reactions of the microbial electrolysis process. Although the voltage supply in MECs is lower than that for water electrolysis, it still consumes huge amount of energy if scale-up the reactors (Manish and Banerjee, 2008). Besides the factors affecting the performance of MFC systems, the applied voltage for hydrogen production by MECs is another key factor, which influences the performance of MECs.

In this chapter, the main objective is to investigate how the impact factors, including substrate concentration, electrode surface area, medium conductivity, membranes and applied voltage, affect the performance of MFCs for both electricity generation and hydrogen production. It has been divided into several minor goals as following:
1. To explore the effect of different substrate concentrations (10mM ~ 80mM) on microbial growth and electricity generation in two-chambered MFCs with two membranes (CEM and PEM).

2. To examine the ability of dual-chambered MFCs for electricity generation as a function of electrode surface areas (14 cm$^2$ ~ 42 cm$^2$) and medium conductivity (9.12 mS/cm ~ 28.0 mS/cm).

3. To investigate the effect of applied voltage (0.3 V ~ 1.05 V) on hydrogen production in two-chamber MECs.

### 3.2 Materials and Methods

#### 3.2.1 Microorganism and cultivation

*Geobacter sulfurreducens* (ATCC 51573) was obtained from American Type Culture Collection (Manassas, VA). The growth medium contained (per liter): 0.1 g of KCl, 0.2 g of NH$_4$Cl, 2.5 g of NaHCO$_3$, 0.6 g of NaH$_2$PO$_4$, 0.82 g of sodium acetate, 10 mL of vitamin mix (ATCC MD-VS), and 10 mL of trace mineral mix (ATCC MD-TMS). The medium was autoclaved and completed with a filtered (0.2 µm) solution of sodium fumarate, and the final concentration of sodium fumarate in the medium was 8 g/L. All the cultivations was carried out under 30 °C (unless stated otherwise).

Batch cultivation of *G. sulfurreducens* was performed using a serum bottle of 250 mL with liquid volume of 200 mL, and *G. sulfurreducens* grown to mid-to-end log phase in batch culture was used as inoculum. After inoculation, each bottle was flushed with a N$_2$-CO$_2$ gas
mixture (80%: 20%, v/v) for 10 minutes to ensure anaerobic condition and sealed with a screwed cap with butyl rubber septum. The inoculum was maintained with the culture medium and stored in an airtight canister with anaerobic generator (Oxoid, AN0035, UK). All transferring and inoculation processes were performed in a biological safety hook by a pipet with sterile tips.

3.2.2 Construction of MFC reactors

The experiments were performed in two-chambered MFCs fed with acetate as substrate. The two-chambered MFC was constructed by two 250 mL glass media bottles (Fisherbrand, FB-800-250, Waltham, MA), which were connected by two glass flanges (diameter of 25 mm, length of 30 mm) (Adams & Chittenden Scientific Glass, NW25, Berkeley, California) and sealed by a sealing ring with clamp (Adams & Chittenden Scientific Glass, NW25, Berkeley, California) as previously described (Kim et al., 2007). There were two sampling ports on the opposite side of the glass flanges on each chamber, and all ports were sealed with open caps and silicone septa. The total geometrical volume of each chamber was 330 mL ± 5mL. The liquid working volume of each chamber, with the electrode, was 270 mL while the headspace was 60 mL.

Non-treated carbon cloth without wet proofing (Fuel Cell Earth, CCP20, Wakefield, MA) was used as anode electrode, while carbon cloth of 40% wet proofing (Fuel Cell Earth, EC4019, Wakefield, MA) coated with platinum of 0.5 mg/cm² as catalyst was used as the cathode electrode. Both electrodes were 3.5 cm × 6.0 cm, and introduced from the top of
bottles by feeding copper wires through the silicone septum (anode chamber) or through a rubber stopper with holes (cathode chamber). New electrodes were soaked in 1 M HCl for 24 hours and flushed with deionized water before using. A membrane with projected area (cross-sectional area) of 4.90 cm$^2$ was held properly by the clamp between the glass flanges and used for the electrolytic contact of the solutions in the two chambers. Before applying to experiment, cation exchange membrane (Membranes International, CMI-7000, Ringwood, NJ) was soaked in 1 M NaCl solution for 24 hours to allow for hydration and expansion. The proton exchange membrane (Dupont, Nafion 117, DE) was pretreated by boiling in H$_2$O$_2$ (30% v/v) and deionized water, followed by soaking in 0.5 M H$_2$SO$_4$ and then deionized water, each for 1 h. Membranes were stored in deionized water prior to use in experiments (Kim et al., 2007).

### 3.2.3 Operation of MFCs

All MFC reactors were cleaned carefully by deionized water, then autoclaved and dried before using. The anode chamber was filled with 250 mL of the medium that was used for bacterium culture without fumarate and inoculated with 20 mL of *G. sulfurreducens* broth, which was grown to mid-to-end log phase in batch culture (after 48 hours culture). Then the anode was aerated with a gas mixture of N$_2$-CO$_2$ (80%: 20%, v/v) for 10 minutes to remove any remaining oxygen, and it was mixed slowly with a magnetic stir bar at 130 rpm. The cathode chamber was filled with 270 mL of 50 mM phosphate buffer at pH=7.0, which was comprised of 0.2918 g/100 mL NaH$_2$PO$_4$•H$_2$O and 0.7733 g/100 mL Na$_2$HPO$_4$•7H$_2$O. The
cathode chamber was provided with air that was passed through the holes of the rubber stopper.

The effect of substrate concentration on the performance of MFC was studied in terms of sodium acetate consumption and power generation. Five levels of sodium acetate concentrations were tested, which were 10mM, 20mM, 40mM, 60mM and 80mM. The effect of electrode area on the performance of MFC was examined with three different electrode areas, which were 14 cm², 28 cm² and 42 cm². The effect of medium conductivity on enhancing electron transfer was explored in terms of power generation by increasing KCl concentration from 0.1 g/L to 5.0 g/L and 10.0 g/L. Two membranes were involved, cation exchange membrane (CEM) and proton exchange membrane (PEM), and each treatment was operated under both CEM and PEM. All MFCs were continuously operated under an external load for 5 days after inoculation and operated at 30°C in open baths with ED heating immersion circulators (Julabo, 9116000, Allentown, PA). When the voltage output was stable, internal resistance of each treatment was analyzed by measuring the voltages under a series of external resistors, which was ranged from 1 kΩ to 100 kΩ (1.0, 1.5, 2.2, 4.7, 6.8, 15.0 and 100.0 kΩ). The subsequent procedure was to remove oxygen from the cathode chamber and add external power supply for hydrogen gas production.

3.2.4 Microbes analysis

The number of bacterial cells was evaluated through the absorbance at 630 nm using a monochromator-based multi-mode microplate reader (BioTek Instrument, Synergy Mx,
Winooski, VT). Absorbance was transformed into total population number per milliliter using the calibration equation of:

total population number = 9 × 10^8 × OD_{630} − 3 × 10^7

which was established through direct observation of cells in a counting chamber slide viewed by a microscope.

### 3.2.5 Chemical analysis

Conductivity was measured using the conductivity cell and meter (Accumet, AP85, Singapore). The acetate was analyzed using a high-performance liquid chromatography (HPLC) (Shimadzu, Model VP, Columbia, MD) equipped with a rezex RHM-Monosaccharide H^+ (8%) column (300 mm × 7.8 mm, 00H-0132-K0, Phenomenex, USA) and a Refractive Index Detector. An aqueous solution of 0.005N H_2SO_4 was used as an eluent solution at 0.6 mL/min, and the column temperature was maintained at 80 °C. Samples were taken by sterile syringes with stainless needles and then filtered through a 0.2 µm pore diameter membrane for analysis.

### 3.2.6 Voltage measurement and H_2 detection

The voltages (E_{cell}) across an external resistor (100 KΩ) in the MFC circuit were monitored in 20 minutes intervals by using a data logger system (DATAQ Instruments, DI-710, Akron, OH). The volume of gas produced in the cathode chamber was measured using a glass syringe of 5 mL capacity (Cadence Science, 5014, Staunton, VA) with a one-way luer-lok stopcock to avoid hydrogen loss when disconnecting syringe. Hydrogen concentration was
analyzed by a gas chromatograph (SRI Instruments, Model 8610C, Menlo Park, California) equipped with a thermal conductivity detector and a molecular sieve column (6’x1/8”, 13X) with nitrogen as the carrier gas.

### 3.2.7 Calculations

Power (P) was calculated according to $P = IE_{MFC}$, where $I$ was the current and $E_{MFC}$ is the voltage across a fixed external resistor (R). The current was calculated from Ohm’s law ($I = E_{MFC}/R$). Thus, power was calculated as

$$P = \frac{E_{MFC}^2}{R} \quad (5)$$

Power is often normalized to some characteristics of the reactor in order to make comparisons to power output of different systems. The power output is usually normalized to the projected anode surface area. The power density ($P_{An}$, W/m$^2$) on the basis of the area of the anode ($A_{An}$) was, therefore, calculated as (Logan et al., 2008).

$$P_{An} = \frac{E_{MFC}^2}{A_{An} \times R} \quad (6)$$

The Coulombic efficiency ($C_E$) is defined as the ratio of the Coulombs actually recovered as current, to maximum possible Coulombs if all substrate removal produced current. The Coulombs actually recovered is determined by integrating the current ($I$) over a period of batch cycle ($t_b$). Thus, the Coulombic efficiency can be evaluated over a period of time as (Liu et al., 2005a)
\[ C_E = \frac{\text{Coulombs recovered}}{\text{Total coulombs in substrate}} = \frac{M_S \int_0^{t_b} I dt}{F b_{ES} V_{An} \Delta c} = \frac{M_S I t_b}{F b_{ES} V_{An} \Delta c} \quad (7) \]

where \( M_S \) is the molecular weight of the substrate, \( F \) is Faraday’s constant (98,485 C/mol of electrons), \( I \) is the current and calculated from Ohm’s law \( (I = E_{MFC}/R) \), \( t_b \) is the time period of a batch cycle, \( b_{ES} \) is the stoichiometric number of moles of electrons produced per mole of substrate \( (b_{ES}=8 \text{ when acetate is used}) \), \( V_{An} \) is the volume of liquid in the anode compartment, \( \Delta c \) is the substrate concentration change over the bath cycle time.

As the cathode is flushed with anaerobic gas prior to the experiment, the volume of hydrogen gas \( (V_{H2}) \) produced is calculated as

\[ V_{H2} = x_{H2} (V_m + V_h) \quad (8) \]

where \( x_{H2} \) is volumetric fraction of hydrogen in a gas sample taken from the headspace, \( V_m \) is the volume of gas produced, which is measured by releasing the gas pressure into a glass syringe, \( V_h \) is the headspace volume.

The amount of hydrogen produced from a substrate is the hydrogen yield. Hydrogen yield for a specific chemical on a molar basis can be calculated as

\[ Y_{H2} \left[ \frac{\text{mol } H_2}{\text{mol } S} \right] = \frac{V_{H2} P M_S}{RT \Delta c_S} \quad (9) \]

where \( \Delta c_S \) (g) is the substrate consumption over a set period of time and \( M_S \) is the molecular weight of the substrate (g/mol), \( P \) (bar) is the atmospheric pressure measured in the laboratory, \( R \) is 0.08314 L bar/K mol.
3.2.8 Internal resistance measurement

The polarization curve is a powerful tool for the analysis and characterization of fuel cells (Hoogers, 2003). A polarization curve represents the voltage (V) as a function of the current (mA) or current density (mA/m$^3$). Polarization curves can generally be divided into three zones: an initial steep decrease of the voltage starting from the OCV; a linear voltage drop with current; a rapid fall of the voltage at higher currents. In the linear zone, the ohmic losses are dominant. Based on the curve of voltages versus current, the value of the internal resistance ($R_{int}$) of the MFC system can be calculated from the equation $R_{int} = -\Delta E/\Delta I$, which represents the slope of the linear zone. The voltages were measured by loading a series of external resistors, which were ranged from 1 kΩ to 100 kΩ (1.0, 1.5, 2.2, 4.7, 6.8, 15.0 and 100.0 kΩ).
3.3 Results and Discussion

3.3.1 Effect of acetate on microbial growth

![Figure 3.1](image)

Figure 3.1 Effect of different initial sodium acetate loadings on *G. sulfurreducens* growth at 30 °C with initial pH at 6.8. The data shown was the means of triplicate samples.

Because acetate is the main substrate degraded by *G. sulfurreducens*, sodium acetate was used as the only electron donor for *G. sulfurreducens* growth. In batch culture of *G. sulfurreducens*, sodium fumarate was used as the electron acceptor in this study, which was fixed at a concentration of 50 mM. Figure 3.1 shows the effect of different sodium acetate...
concentrations on *G. sulfurreducens* growth, and the temperature was controlled at 30 °C with initial pH at 6.8. The standard culture medium of *G. sulfurreducens* contained 0.82 g/L (10mM) of sodium acetate, and the sodium acetate concentration increased from 10 mM to 80 mM with all other chemicals fixed. In this study, the initial optical density for all treatments was fixed at 0.05, which meant that the initial population number of *G. sulfurreducens* in the broth was $1.5 \times 10^7$ (ct.). Based on Figure 3.1, when acetate concentrations increased from 10 mM to 60 mM, the highest optical density (OD) of each test gradually increased from 0.18 to 0.30. The highest final OD (at the 216th hour) of 0.30 was observed in acetate of 20 mM, and the 20mM achieved highest OD within the shortest time among those tests. However, when the acetate was increased to 80 mM, the highest OD was only 0.24, which was lower than the value of 20 mM acetate. Compared to the acetate concentration of 10 mM, all other treatments displayed inhibition effect on *G. sulfurreducens* growth in the first 24 hours, and the inhibition effect became stronger when the acetate increased from 40 mM to 80 mM. In acetate of 80 mM, the microbial growth was strongly suppressed within 120 hours, but the cell concentration (OD) increased after the 120th hour. The inhibition effect on bacterial growth was mainly due to a higher concentration of substrate when the inoculum was fixed at OD of 0.05, and it was released gradually along with the consumption of acetate.
Figure 3.2 shows the consumption of sodium acetate over time by *G. sulfurreducens* with different initial loadings. All sodium acetate in 10mM and 20mM was completely degraded by *G. sulfurreducens*. But the sodium acetate was not completely consumed when the initial loading was up to 40 mM or more. Additionally, for the 40mM, 60 mM and 80mM tests, the consumed sodium acetate was equal to 20 mM. Therefore, 20mM of initial sodium acetate loading was an optimal substrate concentration for *G. sulfurreducens* growth. The result obtained in this study validated by the study of Kim and Lee (2010), and they reported that...
the optimum acetate concentration for \textit{G. sulfurreducens} growth was also 20 mM when the acetate initial loading was ranged from 5 mM to 100 mM.

3.3.2 Effect of acetate concentrations on voltage generation by \textit{G. sulfurreducens}

![Figure 3.3 Voltage generation and substrate degradation as a function of time by \textit{G. sulfurreducens} in a dual-chamber MFC with CEM.](image)

Note: the initial acetate concentration was 10 mM, and cell voltage was measured across a resistor of 100 k\(\Omega\).
Figure 3.3 shows an example of one cycle of voltage generation and acetate consumption as a function of time by *G. sulfurreducens*, and initial sodium acetate loading was 10 mM. In this test, all other conditions were identical with bacterial culture except the electron acceptor. In MFCs, the carbon cloth in anode chamber was used to receive electrons instead of sodium fumarate. From the data shown in Figure 3.3, an abiotic MFC, which functioned as a control test, did not show any significant voltage output. In the MFC inoculated with *G. sulfurreducens*, there was an exponential increase of cell voltage in the first 36 hours, and the voltage was reached 629 mV after 36 hours. The highest voltage output was 681 mV during a period of 120 hours. Concomitantly, the substrate (sodium acetate) was completely degraded at the 48th hour so that the acetate removal of this MFC was 100%. According to the voltage and current outputs, the highest power density normalized to electrode surface area was 13.63 mW/m² and the Coulumbic efficiency was 10.37%.

To further investigate the effect of initial acetate concentrations on the performance of MFCs for electricity generation, different acetate concentrations were fed into the anode chamber, which also increased from 10 mM to 80 mM. In this section, the initial OD after inoculation for all MFCs was also controlled at 0.05 (population number of $1.5 \times 10^7$ ct.). Figure 3.4 and 3.5 show the effects of different acetate concentrations on electricity generation by *G. sulfurreducens* with both CEM and PEM, respectively. From the data shown in Figure 3.4 and 3.5, the cell voltage (across a load of 100 kΩ) was distinguishably increased when the acetate concentration increased from 10 mM to 20 mM or more. In MFCs operated with CEM, the maximum voltage output increased from 650 mV to 824 mV when acetate feeding
increased from 10 mM to 20 mM. While in MFCs operated with PEM, the maximum voltage output increased from 516 mV to 681 mV when acetate feeding increased from 10 mM to 20 mM. The results indicated that the voltage generation gradually increased when substrate concentrations increased. Moreover, the cell voltage in MFCs operated with CEM was greater than that with PEM.

Figure 3.4 Effect of acetate concentrations on voltage generation by *G. sulfurreducens* with CEM.

Note: cell voltage was measured across a load of 100 kΩ.
Figure 3.5 Effect of acetate concentrations on voltage generation by G. sulfurreducens with PEM.

Note: cell voltage was measured across a load of 100 kΩ.

Based on data obtained in MFCs operated with CEMs (Figure 3.4), when the acetate was 20 mM and 40 mM, the cell voltage reached 770 mV at the 36th hour. However, relatively longer adaption periods were observed when the acetate was 60 mM and 80 mM, which took 48 hours to reach 770 mV. In all tests, the voltage outputs were in stationary phase after the adaption period, which was 36 to 48 hours. The values of maximum cell voltages were very
close when the acetate concentration ranged from 20 mM to 80 mM, which were approximate from 824 mV to 834 mV. The current in the MFCs loaded with acetate from 20 mM to 80 mM was not significantly different from each other. The maximum power densities were close, but the Coulumbic efficiency decreased when acetate increased from 20 mM to 80 mM. In the test of 20 mM acetate with CEM, the maximum power density was 79.21 mW/m² and the Coulumbic efficiency was 15.50%, which was higher than the MFC loading with 10 mM acetate.

The identical acetate concentrations of 10 mM to 80 mM were also tested for electricity generation in the MFCs operated with PEM. Figure 3.5 shows the effect of acetate concentrations on voltage generation by *G. sulfurreducens* with PEMs. Consistent with the results obtained in Figure 3.4, cell voltage output increased as the initial acetate loadings increased. The maximum cell voltage increased from 516 mV to 681 mV when acetate feeding was increased from 10 mM to 20 mM. In the MFCs loaded with more than 20 mM acetate, the maximum voltages were not increasingly different, ranging from 667 mV to 681 mV. In the MFCs with 10 to 40 mM of acetate feeding, the cell voltages reached the highest after 36 hours inoculation. However, a longer adaption period of 48 hours was also observed when the acetate was increased up to 60 mM or 80 mM. In MFCs with PEMs, the maximum power density increased from 13.28 to 63.48 mW/m² when the acetate concentration increased from 10 to 20 mM. In terms of Coulumbic efficiency, the Coulumbic efficiency enhanced from 8.79% to 13.86% with the increase of acetate from 10 to 20 mM. These results were in agreement with the results obtained with CEMs in this study, which
demonstrated that electricity generation could be improved by increasing substrate concentration fed in MFCs.

Integrated Figure 3.4 and Figure 3.5, it was found that the maximum cell voltage, maximum power density and Coulombic efficiency were certainly affected by substrate concentrations. When the acetate loading increased greater than 20 mM, cell voltage and power density did not appreciably increase. Integrated with the results obtained in Figure 3.1 and 3.2, bacterial growth did not significantly increase when acetate was greater than 20 mM and no more than 20 mM acetate was degraded by *G. sulfurreducens*. Therefore, the correlation between substrate concentration and electricity generation was demonstrated in this study.

In this study, the maximum power densities were 79.21 mW/m² with CEM and 63.48 mW/m² with PEM, respectively. The results were greater than some studies (Kim et al., 2007; Chae et al., 2009), which used similar reactor architecture and operation parameter with this study. For example, Kim et al. (2007) obtained 38 mW/m² for both Nafion PEM and CEM with 20 mM of acetate loading. Comparing to others, the higher power densities were mainly due to a pure strain inoculation of *G. sulfurreducens* rather than anaerobic sludge inoculation. However, in terms of Coulombic efficiency, the result was lower than some studies (Rabaey et al., 2003; Ren et al., 2007). The maximum current in PEM system with 20 mM of acetate loading was 0.516 mA, and that was 0.577 mA in CEM system with 20 mM of acetate. Accordingly, the Coulombic efficiency CEM system with 20 mM of acetate loading was 15.50% and that was 13.86% in PEM system with 20 mM of acetate. The possible reasons
for the lower Coulombic efficiency comparing with other studies could be the dual-chamber reactor design, substrate loss and oxygen diffusion through membrane (Liu and Logan, 2004; Oh et al., 2004). It has been found that the internal resistance of dual-chamber MFCs was greater than single-chamber MFCs, thus the power density of dual-chamber MFCs was lower (Liu and Logan, 2004a). Because higher internal resistance results in lower current in an MFC system and the Coulombic efficiency was proportional to current in the circuit, the Coulombic efficiency could be increased by reducing internal resistance of an MFC system.

Table 3.1 Properties of membranes and current/Coulombic efficiencies of MFCs.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Membrane properties</th>
<th>Results (acetate of 20 mM, A_{electrode} of 42 cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k_O \times 10^{-4}</td>
<td>Voltag e (mV)\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>k_A \times 10^{-4}</td>
<td>cm/s\textsuperscript{a}</td>
</tr>
<tr>
<td>CEM</td>
<td>0.94</td>
<td>834</td>
</tr>
<tr>
<td>PEM</td>
<td>1.3</td>
<td>664</td>
</tr>
</tbody>
</table>

Note: k_O is oxygen mass transfer coefficient and k_A is acetate mass transfer coefficient. \textsuperscript{a} Referred to Kim et al., 2007. \textsuperscript{b} Obtained in this study and calculated using the formula in section of method and material in this chapter (no repetition).

Compared to the MFCs operated with CEMs, the ones with PEMs showed lower power density and lower Coulombic efficiency in this study. It was observed that the microbial cells
attachment on PEM was more than that on CEM. The attachment of microbial cells on PEMs led substrate loss to cathode chamber, which affected power generation (Oh et al., 2004). Besides, Chae et al. (2008) further investigated PEM (Nafion 117) problems in MFC systems, for example, oxygen leakage, substrate loss, cation transport and accumulation rather than protons. In terms of membrane properties, both oxygen and acetate mass transfer coefficients for PEM (Nafion 117) are relatively greater than CEM (Table 3.1). The loss of substrate and leaking of oxygen have a negative effect on bacterial growth due to insufficient substrate and aerobic conditions. Consequently, the relatively higher mass transfer coefficients of PEM imply the better performance of CEM in electricity generation than PEM, which validated the data obtained in this study.

In conclusion, growth of *G. sulfurreducens* could be affected by different initial substrate feeding concentrations, which also had an effect on electricity generation by dual-chamber MFCs. For electricity generation, the MFC operated with CEM had better performance than that with PEM due to membrane properties. However, the PEMs should be one of the most significant components in MFC systems because of excellent proton conductivity, which would be a key parameter for hydrogen gas production by MECs.

### 3.3.3 Effect of electrode surface area on voltage generation by *G. sulfurreducens*

To further explore the factors affecting the performance of dual-chamber MFCs, the effects of different electrode surface areas on power generation and H$_2$ production were tested with membrane cross-sectional area fixed at 4.90 cm$^2$. The electrode surface areas were designed
by 42 cm$^2$ (3.5cm $\times$ 6.0cm), 28 cm$^2$ (3.5cm $\times$ 4.0cm) and 14 cm$^2$ (3.5cm $\times$ 2.0cm), and anode and cathode areas were kept equal. Table 4.2 shows the results of MFCs as a function of electrode surface areas with both CEM and PEM.

Table 3.2 Performance of MFCs as a function of electrode surface areas.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Electrode Area</th>
<th>$R_{\text{int}}$ (Ω)</th>
<th>Voltage $1\Omega$ (mV)</th>
<th>$PD_{\text{max}}$ (mW/m$^2$)</th>
<th>$I_{\text{max}}$ (mA)</th>
<th>CE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEM</td>
<td>14 cm$^2$</td>
<td>5369</td>
<td>64</td>
<td>6.04</td>
<td>0.064</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td>28 cm$^2$</td>
<td>1663</td>
<td>289</td>
<td>37.09</td>
<td>0.289</td>
<td>7.76</td>
</tr>
<tr>
<td></td>
<td>42 cm$^2$</td>
<td>355</td>
<td>589</td>
<td>82.60</td>
<td>0.589</td>
<td>15.82</td>
</tr>
<tr>
<td>PEM</td>
<td>14 cm$^2$</td>
<td>873</td>
<td>256</td>
<td>46.81</td>
<td>0.256</td>
<td>6.88</td>
</tr>
<tr>
<td></td>
<td>28 cm$^2$</td>
<td>508</td>
<td>413</td>
<td>60.92</td>
<td>0.413</td>
<td>11.09</td>
</tr>
<tr>
<td></td>
<td>42 cm$^2$</td>
<td>401</td>
<td>525</td>
<td>65.62</td>
<td>0.525</td>
<td>14.10</td>
</tr>
</tbody>
</table>

According to the data shown in Table 3.2, it is apparent that the internal resistance of dual-chamber MFCs was affected by electrode surface area. The internal resistance limited the performance of MFCs, such as voltage output, current, power density and Coulombic efficiency. In MFCs with both CEM and PEM, when electrode surface area increased from 14 cm$^2$ to 42 cm$^2$, the internal resistances decreased. Therefore, the power density and Coulombic efficiency increased when the electrode surface area increased. In MFCs operated with CEMs, the maximum power density was observed at electrode surface area of 42 cm$^2$. 
which was 82.60 mW/m$^2$. Correspondingly, the highest Coulombic efficiency was also observed at 42 cm$^2$, which was 15.82%. Moreover, in MFCs operated with PEMs, both maximum power density and highest Coulombic efficiency were also observed at area of 42 cm$^2$, which were 65.62 mW/m$^2$ and 14.10%, respectively. The results indicated that larger electrode surface area resulted in greater current in the circuit and greater cell voltage. Because when the electrode surface area is a limiting factor, larger electrode surface can carry more bacteria and receive more electrons. When the electrons released by bacteria were sufficient in anodic broth, the performance of dual-chamber MFCs was as a function of electrode surface areas, and an increase of electrode surface area could improve the performance of MFCs.

When electrode surface area decreased from 42 cm$^2$ to 14 cm$^2$, the power density decreased from 65.62 to 46.81 mW/m$^2$ in MFCs with PEMs, while that decreased 82.60 to 6.04 mW/m$^2$ in CEMs. The results indicated that the electrode surface area contributed more in the dual-chamber MFCs with CEMs than MFCs with PEMs. This should be attributed to the properties of the membranes and to the contribution of membranes to the internal resistance. Several studies investigated that how the PEMs contributed the internal resistance and affected the performance of MFCs (Oh and Logan, 2006; Liang et al., 2007; Fan et al., 2008). Oh and Logan (2006) demonstrated that the PEM limited the performance (voltage output, power density and internal resistance) of MFCs when PEM cross-section area was smaller than the electrode surface area. Fan et al., (2008) further studied the distribution of each component for the internal resistance, and the result showed that the major contribution of
total internal resistance in two-chamber MFCs was the PEM. The contribution of PEM was distributed 84% to 75% (oxygen as the electron acceptor) when cross-section area of PEM was 6.2-3.5 cm$^2$. Besides, Liang et al. (2007) also demonstrated that the PEM contributed 83% to the total internal resistance of a dual-chamber MFC with a PEM (diameter of 1.5 cm).

Figure 3.6 Effect of electrode surface areas on H$_2$ produced by MECs with CEMs.

Figure 3.6 and 3.7 show the effects of electrode surface areas on H$_2$ produced by MECs with CEMs and PEMs, respectively. According to both figures, it was obvious that the electrode surface area affected the H$_2$ production. In detail, the H$_2$ yield with PEMs decreased from
168.79 to 124.16 mM of H₂/mol of acetate when electrode surface area decreased from 42 cm² to 14 cm², while H₂ yield with CEMs decreased from 119.36 to 53.16 mM of H₂/mol of acetate. Therefore, besides of the effect on power generation by MFCs, the electrode surface area also affected performance of H₂ evolution by MECs. In MECs for H₂ production, the cathodic electrode surface area was the limiting factor, because H₂ was catalyzed by platinum coated on cathodic electrode. Hence, when the protons were sufficient, larger cathodic electrode surface area resulted in higher H₂ yield in MECs.

Figure 3.7 Effect of electrode surface areas on H₂ produced by MECs with PEMs.
The PEM is more selective of proton than CEM, so the proton conductivity of PEM is better than CEM. Better proton conductivity should result in higher H$_2$ gas evolution in MECs. The highest H$_2$ volume and yield were observed at area of 42 cm$^2$ with both CEM and PEM. In the MEC with a PEM and area of 42 cm$^2$, the final H$_2$ volume in headspace was 20.98 mL and H$_2$ yield was 168.79 mM of H$_2$/mol of acetate. In the MEC with a CEM and area of 42 cm$^2$, the final H$_2$ volume in headspace was 14.83 mL and H$_2$ yield was 119.36 mM of H$_2$/mol of acetate. Comparing the MEC with CEM, the MEC with PEM showed higher H$_2$ volume and yield at same electrode surface areas, which was because of a higher proton conductivity of PEM.

3.3.4 Effect of varying medium conductivity on power generation by *G. sulfurreducens*

Besides electrode surface area, the electrolyte resistance contributes to the internal resistance of MFCs. It has been proved that using the ferricyanide as an electron acceptor instead of oxygen could increase the power density (Oh et al., 2004; Oh and Logan 2006), which resulted from a greater mass transfer efficiency using ferricyanide. As additional salts increase electrolyte conductivity, the internal resistance was reduced so that it is beneficial to power generation (ElMekawy et al., 2013). An increase of solution conductivity in anode chamber could increase power generation by MFCs inoculated with anaerobic sludge. In this study, the effects of medium conductivity on power generation and H$_2$ evolution by *G. sulfurreducens* were studied by adding an additional amount of KCl into anode chamber, which changed ionic strength of medium solution.
The culture medium for *G. sulfurreducens* contains 0.1 g/L KCl and the conductivity of the medium is 9.12 mS/cm. The conductivity increases to 17.66 mS/cm when KCl is 5.0 g/L, and it is 28.00 mS/cm with 10.0 g/L of KCl. Table 3.3 shows the results of power generation by *G. sulfurreducens* with an increase of the medium conductivity in dual-chamber MFCs with CEMs. The maximum power density in MFCs with CEMs decreased from 79.21 to 3.75 mW/m² when the KCl concentration increased from 0.1 g/L to 5.0 g/L. For H₂ production by MECs, there was no H₂ gas detected when the KCl was 5.0 g/L or 10.0 g/L in anode chamber, which indicated that proton flux through CEM was too low to form H₂. Because the competition between proton and K⁺ flux through the membrane made few proton went across the membrane. The performance of MFCs with CEMs was limited when additional KCl was added into the medium.

Table 3.3 Performance of MFCs with different medium conductivities under CEMs.

<table>
<thead>
<tr>
<th>KCl (g/L)</th>
<th>Conductivity (mS/cm)</th>
<th>Rₑ (Ω)</th>
<th>Voltage 1kΩ (mV)</th>
<th>PDₘₐₓ (mW/m²)</th>
<th>Iₘₐₓ (mA)</th>
<th>CE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>9.12</td>
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<td>577</td>
<td>79.21</td>
<td>0.577</td>
<td>15.50</td>
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<tr>
<td>5.0</td>
<td>17.66</td>
<td>2514</td>
<td>227</td>
<td>23.15</td>
<td>0.227</td>
<td>6.10</td>
</tr>
<tr>
<td>10.0</td>
<td>28.00</td>
<td>3885</td>
<td>105</td>
<td>3.75</td>
<td>0.105</td>
<td>2.82</td>
</tr>
</tbody>
</table>
Table 3.4 Performance of MFCs with different medium conductivities under PEMs.

<table>
<thead>
<tr>
<th>KCl (g/L)</th>
<th>Conductivity (mS/cm)</th>
<th>R_{int} (Ω)</th>
<th>Voltage 1kΩ (mV)</th>
<th>PD_{max} (mW/m²)</th>
<th>I_{max} (mA)</th>
<th>CE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
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<td>416</td>
<td>536</td>
<td>68.04</td>
<td>0.536</td>
<td>14.40</td>
</tr>
<tr>
<td>5.0</td>
<td>17.66</td>
<td>736</td>
<td>426</td>
<td>53.77</td>
<td>0.426</td>
<td>11.44</td>
</tr>
<tr>
<td>10.0</td>
<td>28.00</td>
<td>1040</td>
<td>362</td>
<td>35.51</td>
<td>0.362</td>
<td>9.72</td>
</tr>
</tbody>
</table>

Table 3.4 shows the results of power generation by *G. sulfurreducens* with an increase of the medium conductivity in dual-chamber MFCs with PEMs. For MFCs operated with PEMs, the power density decreased from 68.04 to 35.51 mW/m² when the KCl concentration increased from 0.1 g/L to 10.0 g/L. For H₂ production, there was also no H₂ gas detected when the KCl was 5.0 g/L or 10.0 g/L in anode chamber.

The trend of the decrease of power density and Coulombic efficiency with an increase of the medium conductivity in this study did not agree with the result obtained in the studies of Liu et al. (2005a) and Oh and Logan (2006), which demonstrated that the power increased as a result of ionic strength increase. In these studies, the anaerobic sludge was inoculated, while pure culture of *G. sulfurreducens* was inoculated in this study. Therefore, the decrease of power when medium conductivity increased should be attributed to two reasons. Firstly, the competition of cations (especially K⁺) and protons in the medium resulted in a decrease of proton transport through CEMs (Choi et al., 2001). The increase of K⁺ concentration by
adding KCl did increase the medium conductivity, however, high concentration of K\(^+\) limited the proton flux and reduction. Secondly, an inhibition effect of high KCl concentration on \textit{G. sulfurreducens} growth (Oh and Logan 2006) resulted in lower substrate consumption rate, which reduced proton production in anode chamber. Comparing to MFCs with CEMs, the lowest power density and Coulombic efficiency obtained in 10.0 g/L of KCl with PEMs were greater. This was still due to PEM was more selective of protons than CEM. In conclusion, when the pure strain of bacterium was inoculated to MFCs, the effect of ionic strength in the medium on bacterial growth should be taken into account for conductivity study.

### 3.3.5 Effect of applied voltage on H\(_2\) production of MECs by \textit{G. sulfurreducens}

In order to investigate the effect of applied voltage on H\(_2\) production by dual-chamber MECs, six levels of external power supply were tested with both CEM and PEM, which were designed as 0.3 V, 0.45V, 0.6 V, 0.75 V, 0.9 V and 1.05V. Figure 3.10 and 3.11 show the H\(_2\) gas volume as a function of applied voltage with CEMs and PEMs, respectively.
Figure 3.10 Effect of different applied voltages on H$_2$ production in MECs with CEMs by $G$. 

$sulfurreducens$. 
Figure 3.11 Effect of different applied voltages on H\textsubscript{2} production in MECs with PEMs by \textit{G. sulfurreducens}.

The H\textsubscript{2} gas production was not detected when the applied voltages were 0.3 V and 0.45 V for both CEMs and PEMs in dual-chamber MECs inoculated with \textit{G. sulfurreducens}. But the theoretical voltage needed to produce H\textsubscript{2} is only 0.130 V, and greater voltages are needed because of the overpotential at the electrode (Liu et al., 2005b). For both CEMs and PEMs, along with the applied voltage increased, the H\textsubscript{2} production increased. This was due to an increase of current applied to the circuit when the applied voltage increased, which provided
more electrons to combine with protons and then improved the hydrogen recovery in the cathode chamber.

For MECs with CEMs, the final (11th day) H$_2$ gas volume produced was 16.04 mL, which was observed under the applied voltage of 1.09 V. In terms of yield based on consumed substrate, it was 129.03 mM of H$_2$/mol of acetate. For the tests under 0.663 V, 0.771 and 0.954 V with CEMs, the final H$_2$ gas volume was similar, which was 12±1.68 mL with an approximate yield of 96.53 mM of H$_2$/mol of acetate.

For the MECs with PEMs, same trend was observed, which was that H$_2$ production increased along with the applied voltage increased. Based on Figure 3.11, the detected H$_2$ volume produced by the 1.09 V was declined after the 8th day, and this was due to a connection failure of the whole circuit. The copper clip connecting wire and electrode was corrupted so that there was an open circuit. The final H$_2$ gas volume produced by the 0.954 V with PEM was 19.96 mL with a yield of 160.56 mM of H$_2$/mol of acetate, which was 66% greater than that obtained with CEM. Besides, as the applied voltage increased from 0.663 V to 0.954 V with PEMs, the final H$_2$ volume increased from 10.45 to 19.96 mL with an increase of hydrogen yield of 84.06 to 160.56 mM of H$_2$/mol. Therefore, the results demonstrated that the H$_2$ production could be affected by varying applied voltage and improved by increasing the applied voltage up to 1.09 V. Additionally, the MECs operated with PEMs outperformed over the MECs with CEMs in terms of H$_2$ production. This should be due to the higher proton conductivity of PEMs.
3.4 Summary and Conclusions

In this study, it has been demonstrated that *Geobacter sulfurreducens* (ATCC 51573) could produce electricity and hydrogen gas in microbial fuel cells (MFCs) by degrading acetate as the electron donor and using carbon cloth as sole electron acceptor. Several key factors were investigated to improve the performance of MFCs, for example, types of membranes, initial substrate loading, electrode surface area, medium conductivity and applied voltage for MECs.

When substrate (sodium acetate) loading was raised from 10 to 20 mM, the power density increased from 13.63 to 79.21 mW/m$^2$ with CEMs and that increased from 13.28 to 63.48 mW/m$^2$ with PEMs. When electrode surface area ($A_{\text{elec}}$) increased from 14 to 42 cm$^2$, the power density increased from 6.04 to 82.60 mW/m$^2$ with CEMs and that increased from 46.81 to 65.63 mW/m$^2$ with PEMs. Moreover, the H$_2$ yield with CEMs increased from 53.16 to 119.36 mM of H$_2$/mol of acetate when $A_{\text{elec}}$ increased from 14 to 42 cm$^2$, and that with PEMs increased from 124.16 to 168.79 mM of H$_2$/mol of acetate. For H$_2$ production by MECs with PEMs, an increase of hydrogen yield from 84.06 to 160.56 mM of H$_2$/mol was achieved with an increase of the applied voltage from 0.663 V to 0.954 V. In addition, an increase of hydrogen yield from 96.53 to 129.03 mM of H$_2$/mol of acetate was observed with when the applied voltage from 0.663 V to 1.090 V.

The MFCs operated with cation exchange membrane (CEM) had better performance of electricity generation than that with proton exchange membrane (PEM). While PEMs outperformed over CEMs for hydrogen gas production by the microbial electrolysis cells.
(MECs). The increase of substrate loading and electrode surface area could improve power generation by MFCs, and the increase of electrode surface area and applied voltage could enhance H₂ evolution by MECs.
REFERENCES


Chapter 4 Electricity and H₂ Generation from Hemicellulose by Binary Culture

Abstract: The main objective in this chapter was to evaluate the performance of MFCs for electricity generation and H₂ evolution by using the acetic acid from anaerobic fermentation as substrate. Preliminary experiment for acetic acid production from corncob hydrolysate by M. thermoacetica was conducted. The acetic acid yield was 0.74 g acid / g xylose when the initial xylose concentration was approximately 10 g/L in corncob hydrolysate under a temperature of 60 °C with an initial pH of 6.8. Then, the second stage was to feed the fermentation product into the anode chamber of MFCs with PEMs for H₂ evolution. For a pure culture of G. sulfurreducens in fermentation broth, the MFCs used an anodic electrode colonized by G. sulfurreducens outperformed over the MFCs inoculated with G. sulfurreducens solution. A power density of 23.20 mW/m² in an MFC and a H₂ yield of 114.36 mM of H₂/mol of acetate in an MEC were obtained, and the MFC/MEC was fed by acetic acid from xylose fermentation and used an electrode colonized by G. sulfurreducens. However, the results of an MFC fed with acetic acid from corncob hydrolysate and used an electrode colonized by G. sulfurreducens were lower, which was due to the inhibition effect of corncob hydrolysate on G. sulfurreducens growth. Besides, the mixed culture of M. thermoacetica and G. sulfurreducens could also produce electricity with a power density of 9.35 mW/m² and generate H₂ with a yield of 31.62 mM of H₂/mol of acetate.
4.1 Introduction

The use of lignocellulose for producing fuels and chemicals has gained increasingly attention because of several advantages of lignocellulose, such as low cost, renewable and widespread (Qu et al., 2006). Cellulosic biomass is one of the most abundant renewable resources, and Departments of Agriculture and Energy estimated the annual availability of biomass feedstock in the United States was 1.3 billion dry tons (Schwarz, 2001). In hemicellulose hydrolysis, xylose is a major sugar component and other sugars, such as glucose and arabinose, are in low amount. Many studies have been investigated the xylitol production by using hemicellulose in agricultural residues, for example, eucalyptus, rice straw, corncob, brewer’s spent grain, sugarcane bagasse, and corn stover (Lynd et al., 2002). Corncob is an attractive raw material for xylose and ethanol conversion (Cai et al., 2012), and xylose can be used as substrate for production of a wide variety of compounds.

By using electrochemically active microorganisms as biocatalysts, microbial fuel cells (MFCs) can bioelectrochemically convert organic material directly into electricity (Logan and Regan, 2006c). Many substrates can be oxidized by different species of bacteria in MFCs, such as acetate, glucose and butyrate. However, most of the electrochemically active microorganisms cannot oxidize xylose directly as a substrate. Therefore, in order to use xylose or hemicellulose material as indirect substrates for electricity generation in MFCs, a two-stage process integrated anaerobic fermentation with microbial fuel cell is an attractive alternative approach. As the acetate is the primary substrate for G. sulfurreducens growth, a microorganism producing acetic acid as major product through fermentation can be
integrated with *G. sulfurreducens* in MFCs. *Moorella thermoacetica* (ATCC 49707) is an improved/mutant strain of *Clostridium thermoaceticum*, and it is a typical strain for acetic acid production and high tolerable for acetic acid accumulation in broth. Thus, *M. thermoacetica* has been chosen for degrading cellulosic or hemicellulosic material to produce acetic acid.

In this chapter, the main objective was to investigate the performance of MFCs for electricity generation and MECs for H₂ evolution by using the acetic acid from anaerobic fermentation as feedstock. Because of the conflict of culture temperatures between *M. thermoacetica* (60 °C) and *G. sulfurreducens* (30 °C), the effect of temperature on performance for acetic acid production by *M. thermoacetica* was studied to. Moreover, the effect of substrate concentration on acetic acid yield by *M. thermoacetica* was investigated. It has been divided into several minor goals as following:

1. To explore the effects of temperatures (30 °C and 60 °C) and initial xylose concentrations (10 g/L, 20 g/L and 30 g/L) on acetic acid production by *M. thermoacetica*.

2. To use corncob hydrolysate, which was pretreated by dilute 1% H₂SO₄ with 10% (w/w) solid loading at 121 °C for 30 minutes, as the fermentation feedstock for acetic acid production by *M. thermoacetica*.

3. To feed the acetic acid, which was fermented from xylose medium or corncob hydrolysate, into anode chamber of MFCs instead of sodium acetate. Both pure culture
and mixed culture of *G. sulfurreducens* and *M. thermoacetica* were explored for electricity generation and H₂ production.

### 4.2 Materials and Methods

#### 4.2.1 Microorganism and cultivation

*Geobacter sulfurreducens* (ATCC 51573) was obtained from American Type Culture Collection (Manassas, VA). Culture method and medium preparation were same as section 3.2.1 in chapter 3. *Moorella thermoacetica* (ATCC 49707) was obtained from American Type Culture Collection, and it is an improved/mutant strain of *Clostridium thermoaceticum*. The seed culture was grown on reinforced clostridial medium (BD 218081) at 60 °C. The seed was transferred to the xylose medium to be acclimatized consuming xylose as carbon source, and then used for further xylose consumption experiments. The xylose medium contained (per liter) 1.0 g of (NH₄)₂SO₄, 0.25 g of MgSO₄·7H₂O, 0.04 g of Fe(NH₄)₂(SO₄)₂·6H₂O, 0.00024 g of NiCl₂·6H₂O, 0.00029 g of ZnSO₄·7H₂O, 0.000017 g of Na₂SeO₃, 0.25 g of cysteine·HCl·H₂O, 5 g of yeast extract, 7.5 g of KH₂PO₄, 4.4 g of K₂HPO₄, 0.415 g of NaOH, 5 g of NaHCO₃ and xylose (Balasubramanian et al., 2001).

The xylose medium for *Moorella thermoacetica* was prepared in five parts and sterilized separately at 121°C for 20 min, then mixed and adjusted pH to 6.8 before inoculation:

i. Mineral solution;

ii. Cysteine·HCl;

iii. Yeast extract;
iv. Buffer (KH$_2$PO$_4$, K$_2$HPO$_4$, NaOH, NaHCO$_3$);

v. Xylose.

All inoculation was performed in biological safety cabinet. The seed of *Moorella thermoacetica* was maintained on both broth and solid (1.5% agar) media, and stored in an airtight canister with anaerobic generator at 4 °C.

### 4.2.2 Acetic acid fermentation by *M. thermoacetica*

For xylose fermentation of *M. thermoacetica*, the medium was same with the xylose medium described above. Batch fermentation from xylose to acetic acid by *M. thermoacetica* was performed with three levels of xylose concentration (10 g/L, 20 g/L and 30 g/L) under two temperature conditions (30 °C and 60 °C) with an initial pH of 6.8. For hemicellulose fermentation of *M. thermoacetica*, corncob hydrolysate and a mimic solution of the hydrolysate were studied for acetic acid production, and the initial pH was also adjusted to 6.8. Each test was performed by triplicate without the pH control.

Before the pretreatment, corncob grinded down to pass a 1 mm screen was used in this experiment. The grinded corncob was pretreated by dilute 1% H$_2$SO$_4$ with 10% (w/w) solid loading at 121 °C for 30 minutes (Cai et al., 2012). The pretreatment was carried out in 500 ml glass flasks, and 20 g of grinded corncob was mixed with 180 mL dilute H$_2$SO$_4$. After autoclaving, the liquid fraction was separated by filtration, and the solid residue was washed
with 40 ml warm water (60 °C) for twice. The filtrate and wash liquid were pooled together and then used as the medium corncob hydrolysate fermentation.

Batch fermentation of *M. thermoacetica* to acetic acid was performed using a serum bottle of 250 mL with medium volume of 200 mL, and 20 mL of *M. thermoacetica* grown to mid-to-end log phase in batch culture was inoculated. Before inoculation, medium was adjusted to pH at 6.8 with 5M HCl, so that there was no significant working liquid volume change in each flask. After inoculation, each flask was flushed with a N₂-CO₂ gas mixture (80%: 20%, v/v) for 10 minutes to develop anaerobic condition and sealed with a screwed cap with butyl rubber septum.

### 4.2.3 MFC inoculation and acclimation

Construction of MFCs was same as section 3.2.2 of chapter 3. Five types of MFCs inoculated with different microorganisms were tested in this study. When the MFCs (MFC-1) were inoculated only with *G. sulfurreducens* (B₁) and filled with culture medium, the anode chambers were fed with 20 mM (1.64 g/L) of sodium acetate without sodium fumarate. The second type of MFCs (MFC-2) were inoculated only with *M. thermoacetica* (B₂) and filled with the xylose medium for fermentation. The third type of MFCs (MFC-3) were inoculated with both *G. sulfurreducens* (B₁) and *M. thermoacetica* (B₂), and filled the electrolyte containing both culture media of B₁ and B₂. The forth type of MFCs (MFC-4) were inoculated only with *G. sulfurreducens* (B₁) and filled with centrifugal xylose fermentation broth, which removed *M. thermoacetica* and contained acetic acid as substrate for *G.*
sulfurreducens instead of sodium acetate. When the cell voltage of MFC-1 stabilized, the anodic electrode was removed from MFC-1 and inserted into the MFCs (MFC-5) contained centrifugal xylose fermentation broth as substrate instead of sodium acetate. And new electrode was then placed into the MFC-1. Therefore, MFC-1, MFC-4 and MFC-5 contained pure culture of G. sulfurreducens (B₁). The difference between MFC-4 and MFC-5 was that MFC-4 was inoculated with G. sulfurreducens (B₁) solution obtained from batch culture, while MFC-5 only contained electrode colonized by G. sulfurreducens (B₁). The MFC-2 contained pure culture of M. thermoacetica (B₂) and MFC-3 contained a co-culture of G. sulfurreducens (B₁) and M. thermoacetica (B₂).

The MFC-4’ and MFC-5’ were similar with MFC-4 and MFC-5, and the difference was MFC-4’ and MFC-5’ contained centrifugal corncob hydrolysate fermentation broth instead of xylose fermentation broth. All transfers were performed aseptically in a biological safety hook, and anaerobic gas was flushed into anode chamber to remove oxygen. The other operation procedures were same as section 3.2.3 in chapter 3.

4.2.4 Microbes analysis

Microbial analysis of G. sulfurreducens was same as section 3.2.4 in chapter 3. Microbial analysis of M. thermoacetica was evaluated through the absorbance at wavelength of 600 nm using a monochromator-based multi-mode microplate reader (BioTek Instrument, Synergy Mx, Winooski, VT).
4.2.5 Chemical analysis

The samples taken from the fermentation broth were filtered through membranes with 0.2 µm pore size and then analyzed for sugar and acetic acid concentrations by high-performance liquid chromatography (HPLC) (Shimadzu, Model VP, Columbia, MD) equipped with a rezex RH-Monosaccharide H⁺ (8%) column (Phenomenex, 300 mm × 7.8 mm, 00H-0132-K0, Torrance, CA) and a Refractive Index Detector. An aqueous solution of 0.005N H₂SO₄ was used as an eluent solution at 0.6 mL/min, and the column temperature was maintained at 80 °C.

4.2.6 Measurements and calculations

The cell voltages (E\text{cell}) across an external resistor (100 KΩ) in the MFCs circuit and volume of H₂ produced in the cathode chamber of MECs were measured by same methods in section 3.2.6 of chapter 3. Further calculations, such as power density and H₂ yield, were conducted by using same formulas in section 3.2.7 of chapter 3.

4.3 Results and Discussion

4.3.1 Effects of temperature and initial xylose concentration on acetic acid yield

In order to investigate the effects of temperature and initial substrate concentration on \textit{M. thermoacetica} growth and acetic acid production, 10 g/L, 20 g/L and 30 g/L of initial xylose concentrations were tested by batch fermentation under both 30 °C and 60 °C with initial pH=6.8. Figure 4.1 and 4.2 show the bacterial growth and pH changes of medium loaded
with different xylose under 30 °C and 60 °C, respectively. In addition, Figure 4.3 and 4.4 show the corresponding xylose consumption and acetic acid production as a function of time.

Figure 4.1 Effects of temperature and initial xylose concentration on microbial growth at initial pH 6.8.
Based on the data shown in Figure 4.1 and 4.2, the final optical density in fermentation broth with different xylose loadings were close under 30 °C, which was approximately 0.77-0.90 (at wavelength of 600 nm), and the final broth pH value was 5.83 ± 0.02. Because the broth pH reflected the acid production by the *M. thermoacetica*, the close broth pH values under 30 °C indicated that the acetic acid production should be approximate either. According to Figure 4.3, the final acetic acid concentrations with different xylose loadings under 30 °C were trivial, which were deceased from 2.045 g/L to 0.435 g/L when xylose loading
increased from 10 g/L to 30 g/L. However, referring to Figure 4.4, almost all xylose was consumed in batch fermentation loaded with 10-30 g/L of xylose under 30 °C. During this experiment (30 °C fermentation), considerable amount of gas was generated, which probably included H₂ and CO₂. Therefore, for fermentation from xylose to acetic acid, temperature of 30 °C was not preferred and favorable by *M. thermoacetica*.

Because the suggested culture temperature given by ATCC is 60 °C for *M. thermoacetica*, the microbes grew much better under 60 °C than 30 °C. According to Figure 4.1, the final optical density was decreased from 1.233 to 0.824 under 60 °C, when initial xylose concentration was increased from 10 to 30 g/L. Subsequently, the final pH value in the broth (Figure 4.2) ranged from 4.91 to 5.25 when xylose loading increased from 10 to 30 g/L under 60 °C. The trend of pH values reflected the acetic acid accumulation in broth, and a lower pH value indicated a lower acetic acid concentration in broth. Therefore, a decrease of acetic acid concentration was expected with the increase of pH value in the broth. Based on the data shown in Figure 4.3, the final acetic acid concentration under 60 °C with 10 g/L of xylose loading was 7.170 g/L in the broth. It was followed by 6.977 g/L of acetic acid with 20 g/L of xylose loading and 5.315 g/L of acetic acid with 30 g/L of xylose loading. It was obvious that the lowest acetic acid concentration under 60 °C was much more greater than the highest acetic acid concentration under 30 °C. Therefore, the culture temperature affects the acetic acid production by *M. thermoacetica*, and the temperature of 60 °C was much more beneficial to acetic acid production from xylose fermentation by *M. thermoacetica*.
Besides the temperature, the initial xylose concentrations also affected the acetic acid production by *M. thermoacetica*. From Figure 4.3, the acetic acid concentration under 60 °C accordantly decreased from 7.170 to 5.315 g/L when initial xylose increased from 10 to 30 g/L. The final acetic acid concentrations under 30 °C also deceased from 2.045 g/L to 0.435 g/L when xylose loading increased from 10 g/L to 30 g/L. Therefore, a higher initial substrate loading could inhibit bacterial growth, then further limit the acetic acid production by *M. thermoacetica*.

Figure 4.3 Effects of temperature and initial xylose concentration on the acetic acid production at initial pH 6.8.
According to Figure 4.4, the initial xylose loading not only affected the acetic acid production by *M. thermoacetica*, but also influenced the concomitant xylose consumption with same amount of bacteria inoculum. Table 4.1 summarizes the effects of temperature and initial xylose loading on acetic acid production by *M. thermoacetica*, including acetic acid yield and xylose consumption rate. The data shown in Table 4.1 indicated that the maximum acetic acid yield was resulted from the 10 g/L of xylose loading under 60 °C, which was 0.91 g acid / g xylose with a xylose consumption rate of 79%. Comparing to fermentation under
60 °C, the tests under 30 °C resulted in significantly less acetic acid yield but great amount of nonessential gas products, although xylose consumption rates were close to 100%. This result was in agreement with the result obtained in Balasubramanian et al. (2001) study. In Balasubramanian’s study, the maximum acetic acid yield of 0.84 g acid / g xylose was observed with 15 g/L of xylose feeding, and the acetic acid yield was also decreased while xylose feeding increased. In conclusion, both temperature and initial xylose loading were limiting factors for acetic acid fermentation by *M. thermoacetica*. The recommended temperature for fermentation from xylose to acetic acid was 60 °C, and the initial xylose feeding of 10 g/L was the optimal among xylose loadings of 10 g/L to 30 g/L.

Table 4.1 Yield of batch fermentation from xylose to acetic acid.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Xylose</th>
<th>Acetic acid</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial loading (g / L)</td>
<td>Consumed (%)</td>
<td>Final concentration (g / L)</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>100</td>
<td>2.054</td>
</tr>
<tr>
<td></td>
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<td>1.400</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>96</td>
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</tr>
<tr>
<td>60</td>
<td>10</td>
<td>79</td>
<td>7.170</td>
</tr>
<tr>
<td></td>
<td>20</td>
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</tr>
<tr>
<td></td>
<td>30</td>
<td>50</td>
<td>5.315</td>
</tr>
</tbody>
</table>
Table 4.1 Continued

Note: the time of batch fermentation was 144 hours.

4.3.2 Acetic acid fermentation by *M. thermoacetica* in mimic medium and corncob hydrolysate.

![Graph showing microbial growth and pH changes with corncob hydrolysate and mimic medium at 60 °C and initial pH=6.8.](image)

Figure 4.5 Microbial growth and pH changes with corncob hydrolysate and mimic medium at 60 °C and initial pH=6.8.
To further investigate the acetic acid conversion from biomass of hemicellulose, the hydrolysate of corncobs was used as the substrate for *M. thermoacetica* growth. Besides hydrolysate medium, a mimic medium was tested as a control, which contained identical saccharides and acids composition with the hydrolysate. As the maximum acetic acid yield was obtained with 10 g/L of xylose feeding, the xylose in corncob hydrolysate was diluted to approximately 10 g/L, and the diluted hydrolysate was used as the medium in this batch of fermentation. Besides, acetic acid production by *M. thermoacetica* with 60 °C was much greater than that under 30 °C, so the fermentation temperature was controlled at 60 °C in this section.

The corncob hydrolysate was pretreated by dilute 1% H₂SO₄ with 10% solid loading at 121 °C for 30 minutes, and the compositions were 2.605 g/L of glucose, 17.286 g/L of xylose, 1.799 g/L of arabinose, 0.099 g/L lactic acid, 2.089 g/L acetic acid, 0.109 g/L butyric acid and 0.064 g/L of butanol. The hydrolysate was diluted to the xylose of approximately 10 g/L, so the other chemicals were subsequently diluted. The mimic medium contained identical amount of chemicals with the corncob hydrolysate except lactic acid, butyric acid and butanol, because the amount of lactic acid, butyric acid and butanol was trivial in the hydrolysate. Therefore, the mimic medium contained 1.724 g/L of glucose, 9.915 g/L of xylose, 2.033 g/L of arabinose, and 1.201 g/L of acetic acid. The pH values of both hydrolysate and mimic medium were adjusted to 6.8 after dilution.
Figure 4.5 shows broth pH value changes and bacterial growth of *M. thermoacetica* in both corncob hydrolysate and mimic medium. The batch fermentation was conducted at 60 °C with an initial pH of 6.8. The final optical density (144th hour) in the mimic medium was 0.943 (OD at 600nm), which was 15.7% higher than that in hydrolysate medium (OD=0.815). In addition, the final broth pH value in the mimic medium was 5.01, which was lower than that of 5.58 in hydrolysate medium. Both OD and pH values indicated that the corncob hydrolysate inhibited the *M. thermoacetica* growth comparing with the mimic medium, which was possibly due to the complex chemical composition in the hydrolysate. The main components of corncob hydrolysate pretreated by dilute H$_2$SO$_4$ were sugars, and there were some inhibitors in a little amount, such as furfural and 5-hydroxymethylfurfural (Cai et al., 2012). The *M. thermoacetica* (ATCC 49707) is a mutant strain of *Clostridium thermoaceticum* and it is high tolerable for acetic acid accumulation in broth. Thus, the lower bacterial density attributed to the inhibitors instead of acetic acid accumulation in the hydrolysate medium.
Figure 4.6 Batch fermentation of *M. thermoacetica* with mimic medium at 60 °C and initial pH=6.8.
Figure 4.7 Batch fermentation of *M. thermoacetica* with corncob hydrolysate at 60 °C and initial pH=6.8.

For acetic acid production, Figure 4.6 and 4.7 show substrates consumption and acetic acid production in both mimic medium and hydrolysate at 60 °C with an initial pH of 6.8. According to both Figure 4.6 and 4.7, the amount of glucose and arabinose did not change significantly. Moreover, along with a decrease of xylose concentration, the concentration of acetic acid increased in the 144 hours of fermentation in both mimic medium and hydrolysate. In the mimic broth, the acetic acid increased from 1.23 g/L to 6.78 g/L with xylose consumed from 8.86 g/L to 1.64 g/L. While in hydrolysate broth, the acetic acid increased from 1.27
g/L to 4.60 g/L with a decrease of xylose from 9.37 g/L to 3.16 g/L. Therefore, it was obvious that *M. thermoacetica* firstly consumed xylose in a mixture of sugars, and xylose mainly contributed to the acetic acid production. The preference of xylose over glucose and arabinose agreed with the result in a study of Balasubramanian et al. (2001). They demonstrated that the xylose consumption rate was faster than that of glucose, arabinose, mannose and galactose.

Table 4.2 Yields of acetic acid from corncob hydrolysate and mimic medium at 60 °C.

<table>
<thead>
<tr>
<th></th>
<th>Xylose</th>
<th>Acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial loading (g / L)</td>
<td>Consumed (%)</td>
</tr>
<tr>
<td>Hydrolysate</td>
<td>9.374</td>
<td>66</td>
</tr>
<tr>
<td>Mimic</td>
<td>8.856</td>
<td>81</td>
</tr>
</tbody>
</table>

Note: the time of batch fermentation was 144 hours.

Table 4.2 summarizes the data of xylose consumption and acetic acid yield for both hydrolysate and mimic medium. In mimic medium, the acetic acid yield was 0.95 g acid / g xylose with a xylose consumption rate of 81%. The results in mimic medium were approximately equal to the results obtained by 10 g/L of xylose loading as sole substrate in section 4.3.1. And it was expected because no inhibitor and unknown chemical were
involved in the mimic medium. In both mimic medium and xylose only medium, the xylose was not completely consumed by *M. thermoacetica*, which may be due to the low pH in broth. In fermentation broth, the pH value was associated with the acid accumulation, and the pH value possibly limited the microbial growth. The acid accumulation in broth could inhibit the substrate consumption, thus the acetic acid production and xylose consumption were correlated with each other. When the pH value was lower than 5.0, the growth of *M. thermoacetica* was limited and no more xylose could be degraded, so acetic acid production stopped.

In corncob hydrolysate, the acetic acid yield was 0.74 g acid / g xylose with a xylose consumption rate of 66%, and the results was lower than the results in mimic medium. The complex of chemical compositions in corncob hydrolysate was a reason for lower acetic acid yield and lower xylose consumption rate. This trend was similar to the results obtained in the tests fed with 20 g/L and 30 g/L of xylose as sole substrate for acetic acid production in section 4.3.1. When the substrate concentration was high or the chemical composition was complicated, the microbial growth was suppressed. In Balasubramanian et al. study (2001), it had been proved that fed-batch operation could not increase acetic acid yield because a loss of microorganism viability after a certain period. From Figure 4.1 and 4.5, the growth of *M. thermoacetica* was in stationary phase after 96 hours of inoculation regardless of the substrate types and concentrations. When the *M. thermoacetica* was fed with a mixture of sugars, there was a 24 hours adaption period. But there was no adaption when only fed with xylose. Therefore, the microorganism viability over a certain period of fermentation was
another reason for no more acetic acid produced in corncob hydrolysate over a longer time. In conclusion, *M. thermoacetica* preferred to oxidize xylose in a mixture of glucose, xylose and arabinose and the corncob hydrolysate could be used as substrate in acetic acid fermentation by *M. thermoacetica*. Besides, when the initial OD after inoculation was controlled at \( \leq 0.2 \), a period of 96 hours was mainly for acetic acid production by *M. thermoacetica*.

4.3.3 Electricity generation of MFCs by pure and mixed cultures

Because optimum substrate concentration for electricity generation by *G. sulfurreducens* was 20 mM of sodium acetate, the fermentation broth contained acetic acid was diluted to acetic acid concentration of 20 mM and then adjusted to pH of 6.8. The diluted fermentation broth with a pH of 6.8 was filled into anode chamber as the electron donor for *G. sulfurreducens*. There were five types of MFCs/MECs in this section of experiments as described in 4.2.3.
Figure 4.8 Cell voltage generated by MFC-1, MFC-2 and MFC-3 with PEMs (Cell voltage was measured across a load of 100 kΩ).

Figure 4.8 shows the cell voltages of MFC-1, MFC-2 and MFC-3 with PEMs. MFC-1 was inoculated with *G. sulfurreducens* only and fed with culture medium of *G. sulfurreducens* loaded by 20 mM of sodium acetate. In MFC-1, the cell voltage stabilized at 671 mV during 66 hours inoculation, and then a new clean electrode was transferred into the anode chamber. The cell voltage rapidly increased to 694 mV within 6 hours of replacement as the new electrode was quickly colonized by *G. sulfurreducens*, which had grown to stationary phase in anode broth. With new anodic electrode in MFC-1, the cell voltage stabilized at 696 mV.
MFC-2 was inoculated with *M. thermoacetica* only to test whether *M. thermoacetica* could produce electricity. An unchanged cell voltage of MFC-2 was observed, and it was fixed at less than 200 mV, which was trivial and observed as default voltage output of xylose medium for *M. thermoacetica*. MFC-3 contained a mixed culture of *G. sulfurreducens* and *M. thermoacetica* at 30 °C, and the cell voltage reached 659 mV at 42 hours of inoculation. The adaption period of voltage stabilization in a mixed culture was 6 hours longer than pure culture of *G. sulfurreducens* in MFC-1, which was 42 hours. The maximum voltage output of mixed culture (MFC-3) was 684 mV, which was extremely close to pure culture of *G. sulfurreducens* (MFC-1).
Figure 4.9 Cell voltage generated by MFC-1, MFC-4 and MFC-5 with PEMs (Cell voltage was measured across a load of 100 kΩ).

Figure 4.9 shows the cell voltages of MFC-1, MFC-4 and MFC-5 with PEMs. MFC-4 was inoculated with pure strain of *G. sulfurreducens* and filled with centrifugal xylose fermentation broth obtained from experiment of section 4.3.1, which contained acetic acid fermented from xylose but without *M. thermoacetica*. MFC-5 also contained centrifugal xylose fermentation broth as substrate, but the anode was the anodic electrode removed from MFC-1, which was colonized by *G. sulfurreducens*. The difference between MFC-1 and MFC-4 or MFC-5 was the substrate. MFC-1 was fed with sodium acetate, while MFC-4 was
fed with the broth of xylose fermentation. The cell voltage of MFC-4 reached the 662 mV after 48 hours of inoculation, which had a 12-hour longer adaption period than MFC-1. And the maximum cell voltage of MFC-4 was 684 mV, which was same to the maximum cell voltage of MFC-1. In MFC-5, after inserting electrode already colonized by *G. sulfurreducens*, the cell voltage dropped to 719 mV after 12 hours of inoculation, and it was kept at approximately 720 mV thereafter. And the voltage obtained in MFC-5 was comparable to the voltage obtained in MFC-1 and MFC-4. According to the results in MFC-4 and MFC-5, the centrifugal xylose fermentation broth can be used by *G. sulfurreducens* as the substrate in MFCs for electricity generation. Besides, based on the result in MFC-5, the microbial cells attached on the electrode surface contributed to the electrons transfer and electricity generation.
Figure 4.10 Cell voltage generated by MFC-4’ and MFC-5’ with PEMs (Cell voltage was measured across a load of 100 kΩ).

Figure 4.10 shows cell voltage generated by MFC-4’ and MFC-5’. MFC-4’ and MFC-5’ were filled by centrifugal corncob hydrolysate fermentation broth obtained from the experiment of section 4.3.2 rather than centrifugal xylose fermentation broth. The cell voltage of MFC-4’ reached 373 mV with an adaption period of 30 hours, and it was stabilized at 386 mV. MFC-5’ used the anodic electrode removed from MFC-1 and colonized by pure *G. sulfurreducens*, and it was also filled by centrifugal corncob hydrolysate.
fermentation broth. The cell voltage of MFC-5’ reached 677 mV after 30 hours of inoculation and stabilized at 673 mV thereafter.

Comparing to MFC-1, the cell voltages of MFC-3, MFC-4, MFC-5 and MFC-5’ were close to the cell voltage of MFC-1, which was approximately 670-680 mV. However, the cell voltage of MFC-4’ (386 mV) was significantly lower than ~670 mV. The MFC-4’ was inoculated by pure *G. sulfurreducens* broth, and the corncob hydrolysate fermentation broth was used as substrate for *G. sulfurreducens*. As concluded in 3.3.2, the growth of *G. sulfurreducens* was correlated with the electricity generation in MFCs, the lower cell voltage of MFC-4’ was because of the lower growth of the microbes. The complex chemicals in corncob hydrolysate not only inhibited the growth of *M. thermoacetica*, but also inhibited the *G. sulfurreducens*. In contrast, the MFC-5’ also used the corncob hydrolysate fermentation broth as substrate, and the cell voltage was closed to the result of MFC-1. This was because the anodic electrode of MFC-5’ was fully colonized by *G. sulfurreducens* before inserting into MFC-5’. In conclusion, the product of hemicellulose fermentation could be used as substrate for electricity generation by *G. sulfurreducens* in MFCs, and using an anodic electrode already colonized by *G. sulfurreducens* had better performance. This can be validated by several studies (Lower et al., 2001; Gorby and Beveridge, 2005). Gorby and Beveridge (2005) demonstrated that nanowires produced by *G. sulfurreducens* resulted in the high efficiency of electrons transfer. And Lower et al. (2001) stated the extracellular cytochrome of *G. sulfurreducens* was responsible for electrons transfer through the
attachment on electrode. Therefore, in the MFCs fed by hemicellulose fermentation broth, using an anodic electrode already colonized by *G. sulfurreducens* had better performance.

### 4.3.4 H₂ production of MECs by pure and mixed cultures

Table 4.3 Performance of MFCs/MECs with pure and mixed cultures under PEMs.

<table>
<thead>
<tr>
<th></th>
<th>Voltage&lt;sub&gt;1kΩ&lt;/sub&gt; (mV)</th>
<th>PD&lt;sub&gt;max&lt;/sub&gt; (mW/m²)</th>
<th>CE (%)</th>
<th>H₂ Yield (mM of H₂/mol of acetate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFC-1</td>
<td>536</td>
<td>68.04</td>
<td>14.40</td>
<td>168.79</td>
</tr>
<tr>
<td>MFC-3</td>
<td>172</td>
<td>9.35</td>
<td>4.6</td>
<td>31.62</td>
</tr>
<tr>
<td>MFC-4</td>
<td>269</td>
<td>19.89</td>
<td>7.2</td>
<td>41.74</td>
</tr>
<tr>
<td>MFC-5</td>
<td>288</td>
<td>23.20</td>
<td>7.74</td>
<td>114.36</td>
</tr>
<tr>
<td>MFC-4’</td>
<td>168</td>
<td>6.82</td>
<td>4.5</td>
<td>23.25</td>
</tr>
<tr>
<td>MFC-5’</td>
<td>184</td>
<td>10.74</td>
<td>4.9</td>
<td>77.26</td>
</tr>
</tbody>
</table>

Table 4.3 summarizes the performance of the MFCs/MECs fed by different substrates with a pure or a mixed culture with PEMs. According to the data shown in Table 4.3, the H₂ production was corresponded with electrons transfer and related to the results of power generation in each type of MFCs. In terms of both electricity and H₂ generation, MFC-1 showed the best performance. It was because that a pure strain of *G. sulfurreducens* was in a standard culture condition in MFC-1. Among the other MFCs, the MFC-5 and MFC-5’
showed the higher results than the MFC-3, MFC-4 and MFC-4’, for both electricity generation and H\textsubscript{2} production. The better performance of MFC-5 and MFC-5’ was resulted from an anodic electrode colonized by pure \textit{G. sulfurreducens}. The electrode colonized by pure \textit{G. sulfurreducens} could reduce the inhibition effect of fermentation broth on growth of \textit{G. sulfurreducens}. Therefore, a pre-grown \textit{G. sulfurreducens} on electrode or a longer adaption period of \textit{G. sulfurreducens} in the fermentation broth was required when feeding hemicellulose fermentation broth as substrate into MFCs.

4.4 Summary and Conclusions

\textit{Moorella thermoacetica} (ATCC 49707) is an improved/mutant strain of \textit{Clostridium thermoaceticum}, and it is high tolerable for acetic acid accumulation in broth. \textit{M. thermoacetica} can produce acetic acid by degrading xylose as a primary substrate. Fermentation temperature and the initial xylose concentrations affected the acetic acid yield by \textit{M. thermoacetica}, and an increase of xylose loading did not enhance the bacterial growth or the acetic acid production. A recommended temperature for the fermentation from xylose to acetic acid by \textit{M. thermoacetica} was 60 °C, and the highest yield of 0.91 g acid / g xylose with a xylose consumption rate of 79% was resulted from an initial xylose feeding of 10 g/L. Further study of acetic acid conversion from hemicellulose was conducted, the hydrolysate of corncobs was used as substrate for \textit{M. thermoacetica}. The corncob hydrolysate contained a mixture of sugars, such as glucose, xylose and arabinose. An acetic acid yield of 0.74 g acid / g xylose was obtained in corncob hydrolysate, and the xylose concentration in the corncob hydrolysate was to10 g/L.
The fermentation product, acetic acid, was fed into anode chamber of MFCs as a substrate for electricity generation and H₂ production. The MFCs were inoculated with both pure culture and mixed culture of *G. sulfurreducens* (B₁) and *M. thermoacetica* (B₂). The result showed that a pure culture of *M. thermoacetica* did not generate any current in an MFC (MFC-2), hence there was no H₂ detected in a corresponding MEC. The MFC (MFC-3) contained a mixed culture of B₁ and B₂ at 30 °C generated a cell voltage of 673 mV, with a power density of 9.35 mW/m² and a H₂ yield of 31.62 mM of H₂/mol of acetate. By feeding the acetic acid from xylose fermentation into MFCs, a power density of 23.20 mW/m² was resulted from using an electrode colonized by B₁ (MFC-5), and a power density of 19.89 mW/m² was resulted from inoculating B₁ broth into anode chamber (MFC-4). When MFCs was fed by acetic acid from corncob hydrolysate, a power density of 10.74 mW/m² was observed by using an electrode colonized with B₁ (MFC-5’), and a power density of 6.82 mW/m² was observed by inoculating B₁ into anode chamber (MFC-4’). Accordingly, the results of H₂ production were strongly in agreement with trend of power generation. In terms of the substrates, the H₂ yield in the MECs fed with xylose fermentation broth was higher than the MECs fed with corncob fermentation broth. When feeding by the same type of fermentation broth, the MECs operated with electrodes colonized with B₁ had higher H₂ yields than the MECs inoculated with B₁ broth.

Therefore, the acetic acid from both xylose fermentation and corncob hydrolysate can be used as substrate for *G. sulfurreducens* in MFCs/MECs. The electrons transfer was not only due to the extracellular cytochrome of outer cell membrane through attachment on electrode,
but also due to nanowires produced by *G. sulfurreducens* resulting in electrons transfer from surrounding electrolyte to electrode.
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Chapter 5 Conclusions and Recommendations

5.1 Conclusions

The performance of electricity generation and H₂ evolution by microbial fuel cells was evaluated in this study. The following conclusions were drawn:

1. *Geobacter sulfurreducens* (ATCC 51573) could produce electricity and hydrogen gas in microbial fuel cells (MFCs) by degrading acetate as a substrate.
   
a) When substrate (sodium acetate) loading was raised from 10 to 20 mM, the power density increased from 13.63 to 79.21 mW/m² in MFCs with CEMs and increased from 13.28 to 63.48 mW/m² in MFCs with PEMs. The corresponding Coulumbic efficiency with CEMs increased from 10.37% to 15.50% and increased from 8.79% to 13.86% with PEMs if sodium acetate increased from 10 to 20 mM.

b) When electrode surface area (A_{elec}) increased from 14 to 42 cm², the power density increased from 6.04 to 82.60 mW/m² with CEMs and that increased from 46.81 to 65.63 mW/m² with PEMs. Moreover, the H₂ yield with CEMs increased from 53.16 to 119.36 mM of H₂/mol of acetate when A_{elec} increased from 14 to 42 cm², and that with PEMs increased from 124.16 to 168.79 mM of H₂/mol of acetate.

c) For H₂ production by MECs with PEMs, an increase of hydrogen yield from 84.06 to 160.56 mM of H₂/mol was achieved with an increase of the applied voltage from 0.663 V to 0.954 V. In addition, an increase of hydrogen yield from 96.53 to 129.03 mM of H₂/mol of acetate was observed with PEMs when the applied voltage from 0.663 V to 1.090 V.
2. The MFCs operated with cation exchange membrane (CEM) had better performance of electricity generation than that with proton exchange membrane (PEM). While, PEMs outperformed over CEMs for hydrogen gas production by the microbial electrolysis cells (MECs).

3. A pure culture of *Moorella thermoacetica* (ATCC 49707) could not generate electricity in MFCs, and no production of H₂ in MECs. However, a mixed culture of *G. sulfurreducens* and *M. thermoacetica* inoculated into MFCs showed a power density of 9.35 mW/m² and a H₂ yield of 31.62 mM of H₂/mol of acetate.
   a) The recommended temperature for acetic acid fermentation by *M. thermoacetica* was 60 °C, and a yield of 0.91 g acid / g xylose with a xylose consumption rate of 79% was resulted from an initial xylose feeding of 10 g/L. When the corncob hydrolysate (contains 10 g/L of xylose) was used as feedstock in acetic acid fermentation by *M. thermoacetica*, the acetic acid yield was 0.74 g acid / g xylose.
   b) The MFCs fed by xylose fermentation broth had better performance of electricity generation and H₂ production than feeding by the corncob hydrolysate fermentation broth, which was because of the inhibitors in corncob hydrolysate on *G. sulfurreducens* growth.
   c) For a pure culture of *G. sulfurreducens* in the fermentation broth, the MFCs/MECs used an anodic electrode colonized by *G. sulfurreducens* outperformed over the MFCs inoculated with *G. sulfurreducens* solution. It indicated that the electrons transfer was mainly contributed to the attachment of bacterial cells on electrode.
5.2 Recommendations

The following are recommended for future studies:

1. Microbial fuel cells or microbial electrolysis cells reactors of similar design will be studied to optimize operational conditions in the future. The suggested substrate concentration for *G. sulfurreducens* will be 20 mM sodium acetate with a relatively lower concentration of phosphate buffer, such as 50 mM.

2. The anodic electrode material with a large and rough surface area is recommended for future studies, because the attachment of microbial cells on the electrode is generally responsible for electron transfer within the system. With future advances in material science, it will be possible to replace the platinum coating on the cathodic electrode using other lower cost catalysts, which will make the MFCs more cost-effective in practice. As these materials become available, it will be important to test and optimize the operational conditions using the new electrodes.

3. When future research focuses on bio-hydrogen production (as opposed to generation of electricity) by microbial electrolysis cells, a proton exchange membrane is recommended.

4. Acetic acid fermentation in batch mode by *M. thermoacetica* should be studied in the future. Suggested starting temperature for experimental trials is 60 °C and the suggested substrate concentration is 10 g/L if xylose is used as a substrate.

5. For future trials to study a two-stage process integrating anaerobic fermentation within microbial fuel cells for electricity generation and H₂ production, the suggested inoculation method is to pre-attach or pre-grow the *G. sulfurreducens* on the anodic
electrode material first, then use the electrode colonized with *G. sulfurreducens* in the MFCs to be studied.

5.3 Contribution of This Study and Future Work

This study was the first research project in the field of microbial fuel cells in Dr. Wenqiao Yuan’s group. It provides an excellent beginning for future related research because it demonstrates several fundamental concepts related to MFCs and validates methods useful for analyzing the performance of microbial fuel cells. Future work in this research area should address optimization of the reactor architectures. One of most critical steps for future research will be to scale-up the microbial fuel cell systems in practice and to perform life cycle assessments for the larger scale systems. This future information will lead to development and evaluation of cost-effective materials for the reactor and will potentially enhance the power output of MFCs or H₂ yield in the future.