ELECTRICITY PRODUCTION FROM CARBON MONOXIDE AND SYNTHESIS GAS IN A MICROBIAL FUEL CELL

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ABSTRACT

Synthesis gas (syngas), which primarily consists of carbon monoxide (CO) and hydrogen (H_2), is a versatile energy carrier that can be converted to gaseous and liquid fuels or can be used for electricity production. While the ability of CO and syngas to serve as sole electron donors for electricity production in a microbial fuel cell (MFC) was recently demonstrated, this study has focused on MFC design improvements for performance enhancement on CO/syngas and elucidation of microbial communities, and biotransformation pathways involved in electricity production from CO/syngas in an MFC.

One of the primary challenges for an efficient bioconversion of CO and syngas is the low solubility of these gaseous substrates in the aqueous phase. The first study of this thesis demonstrated the applicability of silicone membrane systems for improved CO transfer into the anodic liquid of MFCs. The incorporation of flat silicone membrane and thin wall silicone tubing into the anodic chamber of CO-fed MFCs led to improved CO transformation efficiency and correspondingly improved MFC performance. A CO transformation efficiency of 77 % and maximum power output of 18 mW L_R^{-1} (normalized to anodic compartment volume) was achieved for silicone membrane installed MFC. A comparably higher CO transformation efficiency of 98 % was obtained for silicone tubing installed MFC, but the high dissolved CO concentrations in the anodic liquid partially inhibited the microbial activity, thereby lowering the maximum power output to 13 mW L_R^{-1} .

Efficient gas transfer also allowed for focusing on the process microbiology. The microbial communities and biotransformation pathways prevalent in two mesophilic CO-fed MFCs were elucidated in the second study. The identification of the microorganisms

belonging to the genera *Geobacter*, *Desulfovibrio*, and *Clostridium*, along with the detection of acetate as the primary metabolic product in both MFCs; affirmed our hypothesis that electricity production from CO/syngas in a mesophilic MFC is primarily accomplished by a two-step process, where CO/syngas is first converted to acetate by homo-acetogenic and carboxdotrophic microorganisms, and the acetate is then utilized by CO-tolerant acetate oxidizing electricigenic microorganisms.

Considering that syngas is primarily a hot gas, the bioconversion of CO/syngas to electricity in an MFC was also tested at thermophilic temperature of 50 °C. Silicone tubing was used for syngas delivery and the anodic design was improved to increase the microbial density. An improved volumetric power output of 33-35 $mW\,L_R^{-1}$ and syngas conversion efficiency of 87-98 % was achieved. Also an improved Coulombic efficiency (CE) of 26 % was obtained. The analysis of the anodic microbial communities and metabolic products, along with single substrate tests where MFC was operated solely on CO or H_2, revealed that similar to mesophilic MFCs electricity generation from syngas at thermophilic temperatures also occurred through syngas conversion to acetate followed by its oxidation by CO-tolerant electricigenic microorganisms.

In the final study of this thesis, the applicability of a multi-electrode design containing three anodes and two cathodes to achieve high volumetric power output and CE on syngas was evaluated at several operating temperatures ranging from 37 °C to 50 °C. Also, the impact of different anode-cathode arrangements on power output was examined. The multi-electrode configuration considerably enhanced the system performance and provided a compact system design which could have major economic and operational implications for large scale syngas-fed MFC systems. A maximum power density of 33 $mW\,L_R^{-1}$ and CE of

43 % was achieved at an operating temperature of 37 °C. The MFC performance at elevated temperatures was restricted by low thermophilic microbial activity. Consequently, a much lower power density of 10 $mW\,L_R^{-1}$ and a CE of 15 % were obtained at 50 °C. The MFC power density was greatly impacted by the anode-cathode arrangement and the highest power density was achieved in a three anode-two cathode (3A-2C) arrangement.

The findings presented in this thesis could be utilized for further improvements in the reactor design and biological system of CO/syngas-fed MFCs for making such contraptions suitable for commercial applications. An MFC based process could also be used to combine electricity production with hydrogen extraction from syngas or in a combination with syngas transformation to valuable fermentation products.

RÉSUMÉ

Composé principalement de monoxyde de carbone (CO) et d'hydrogène (H_2), le gaz de synthèse est un vecteur énergétique polyvalent, qui peut être converti en un combustible liquide ou gazeux ou peut servir à la production d'électricité. La capacité du CO ou de gaz de synthèse à servir comme unique donneur d'électrons dans la production d'électricité dans une pile à combustible microbienne (PCM) ayant été récemment démontrée, la présente étude visa l'obtention d'un gain d'éfficacité de production, avec soit du CO ou du gaz de synthèse comme combustible, grâce à un aménagement amélioré de la PCM. L'étude visa aussi à élucider les communautés microbiennes et voies de biotransformation liées à la production d'électricité à partir de CO ou de gaz de synthèse dans une PCM.

Un des principaux défis à une hausse d'efficacité de bioconversion de CO ou de gaz de synthèse est la faible solubilité en phase aqueuse de ces substrats gazeux. Le premier volet de ce travail démontra l'applicabilité de systèmes à membrane de silicone pour réaliser un transfert accru de CO vers l'anode liquide du PCM. L'intégration d'une membrane plane en silicone ou de tube en silicone à parois minces dans la chambre anodique d'une PCM alimentée en CO, a permis d'atteindre une éfficacité de transformation de CO accrue, et par conséquente une performance améliorée de la PCM. Une efficacité de transformation de CO de 77% et une puissance maximale de 18 $mW\,L_R^{-1}$ (normalisée selon le volume de la chambre anodique) furent obtenues pour une PCM équipée d'une membrane en silicone. Une efficacité de transformation de CO comparativement plus élevée (98%) fut obtenue pour une PCM équipée de tube en silicone à parois minces, mais la concentration éleve de CO dissoute dans le liquide anodique enraya en partie l'activité microbienne, baissant ainsi la puissance maximale à 13 $mW\,L_R^{-1}$.

Un transfer gazeux efficace permetta de mettre l'accent sur le processus microbiologique. En un second volet, les communautés microbiennes et voies de biotransformation prévalentes dans deux PCM mésophiliques alimentées en CO furent élucidés. L'identification de microorganismes appartenants aux genres *Geobacter*, *Desulfovibrio*, et *Clostridium*, ainsi que la détection d'acétate comme principal produit métabolique dans les deux PCM, confirma notre hypothèse que la production d'électricité à partir de CO ou de gaz de synthèse dans une PCM mésophilique s'opère principalement en deux étapes: (i) le CO ou gaz de synthèse est premièrement converti en acétate par des carboxydobactéries et bactéries homoacétogéniques, puis (ii) l'acétate est oxydé par des microorganismes électrigènes tolérants au CO.

Le gaz de synthèse étant principalement un gaz chaud, la bioconversion de CO ou de gaz de synthèse dans une PCM fut évalue sous des condition thermophiles à 50 °C. Le tube de silicone servant à livrer le gaz de synthèse et l'aménagement de l'anode furent améliorés afin d'augmenter la densité microbienne. Une puissance volumétrique accrue (33-35 $mW\,L_R^{-1}$) et une éfficacité de conversion de gaz de synthèse de 87-98% furent atteintes. De plus une efficacité coulombienne (EC) de 26% fut atteinte. Une analyse des communautés microbiennes anodiques et de leur métabolites, ainsi que des essais à substrat unique, où les PCM fonctionnèrent avec du CO ou du H_2 seulement, démontrèrent que, semblable à la production d'électricité par des PCM mésophiles, la production d'électricité à partir de gaz de synthèse à des températures thermophiles opéra par conversion du gaz de synthèse en acétate, suivi de son oxydation par des microorganismes électrigènes tolérants au CO.

Dans le dernier volet de cette étude, la faisabilité d'une configuration à multiples électrodes (3 anodes, 2 cathodes) visant à permettre des PCMs consommant du gaz de synthèse à obtenir une puissance volumétrique et une EC accrue, fut évaluée à des

températures de 37 °C à 50 °C. L'influence de la position dans laquelle les anodes et cathodes furent disposées sur la puissance générée fut évalue. Une configuration multi-électrode augmenta considérablement la performance du système et donna lieu à un système compact pouvant avoir d'importantes implications opérationnelles et économiques pour des systèmes PCM à grande échelle consommant du gaz de synthèse. Une densité de puissance maximale de 33 m^{-1} L_R^{-1} et une CE de 43 % furent atteintes à une température de fonctionnement de 37 °C. La performance de la PCM à de plus hautes températures fut limitée par la faible activité des microbes thermophiles. Consequemment, une densité de puissance beaucoup plus faible de 10 mW L_R^{-1} et une CE de 15% furent obtenues à 50 °C. La densité de puissance d'une PCM fut largement influencée par la disposition et le nombre des anodes de cathodes. Un arrangement trois anodes-deux cathodes (3A-2C) offrant la plus grande puissance.

Les présents constats pourraient servir à l'amélioration de l'aménagement de tels reacteurs et du système biologique de PCMs ayant du CO ou du gaz de synthèse comme combustible. Un procédé opérant avec une PCM pourrait aussi servir à combiner la production d'électricité avec l'extraction d'hydrogène du gaz de synthèse, ou en combinaison avec une transformation du gaz de synthèse en produits de fermentation ayant une valeur ajoutée.

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CONTRIBUTIONS OF THE AUTHORS

Abid Hussain is the principle author of this work which was supervised by Dr. Boris Tartakovsky from the Biotechnology Research Institute (BRI), National Research Council of Canada, Montréal, Québec, and Dr. Vijaya Raghavan from the Department of Bioresource Engineering, McGill University, Ste-Anne-de-Bellevue, Québec, Canada. The entire experimental work was performed at the Environmental Bioengineering facility at BRI. Ms. Punita Mehta and Dr. Guillaume Bruant from BRI were personally involved in giving valid suggestions in every stage of this study and provided technical assistance for microbiological studies. Dr. Serge Guiot from BRI provided expert guidance and made immense contribution in reviewing and improving the writing of the manuscripts mentioned below.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

General Introduction

1.1 Motivation

A steady increase in energy consumption in developed countries and a surge in energy demands in the fast growing developing economies might lead to a shortage of fossil fuels in a foreseeable future. This anticipated shortage, along with the confluence of concerns about atmospheric pollution and climate change are acting as a major impetus for research into alternative renewable energy technologies. A number of studies have suggested biomass to be one of the most promising sources of renewable energy (Kim & Chang, 2009; Song, 2002). Microbial digestion, fermentation and gasification are well known processes, among many, for biomass conversion to biofuels and bioproducts. Wet biomass with up to 25-35% of solids such as urban organic waste and high moisture agricultural wastes (vegetables, sugar cane, sugar beet, etc) are best suited for microbial degradation, while gasification is most appropriate for dry biomass such as woody wastes and low moisture agricultural wastes (Demirbas, 2001; Demirbas, 2007).

The gasification of biomass at high temperatures leads to the generation of synthesis gas (syngas). Carbon monoxide and hydrogen account for 60-80% of the syngas composition, with CH_4, CO_2, SO_2, H_2S and NH_3 present in smaller amounts (Munasinghe & Khanal, 2010; Sipma et al., 2006). Although most of the syngas today is produced from non-renewable sources, such as natural gas and coal, syngas production from biomass or poorly

degradable organic matter makes syngas generation an environmentally friendly option for energy production (Faaij et al., 1997; Henstra et al., 2007). Syngas represents a versatile energy carrier which can be used as a chemical feedstock to produce liquid and gaseous fuels such as ethanol, methane, etc. or can be directly utilized for power production through combustion. However, the low conversion efficiency of the internal combustion engines and the generation of soot and sulfur compounds, in addition to other noxious emissions present the major drawbacks of the process.

There are many studies focusing on utilization of CO or syngas for electricity generation in fuel cells (Baschuk & Li, 2001; Ormerod, 2003; Song, 2002; Steele & Heinzel, 2001). Unlike combustion that converts chemicals to reaction products (e.g., H_2O and CO_2) and heat, fuel cells directly produce electrical energy by electrochemical oxidation of the fuel. Such a chemical process of electricity generation confers fuel cells with the intrinsic advantage of high electrical efficiency and low environmental impact. CO/syngas can be potentially used in polymer electrolyte membrane fuel cells (PEMFC) and solid oxide fuel cells (SOFC). However, efficient utilization of CO/syngas is hindered due to the sensitivity of metal catalysts used in such devices to CO and/or trace impurities in the syngas stream such as sulfur. Platinum (Pt) or platinum based alloys used in PEMFC are extremely sensitive to CO and sulfur compounds with more than 10 ppm of CO and 0.1 ppm of sulfur compounds resulting in irreversible poisoning and inhibition of the catalyst (Kim & Chang, 2009; Ormerod, 2003). Similarly, SOFCs which generally employ nickel as the anode are easily poisoned by the presence of more than 10 ppm of sulfur compounds. The sulfur compounds are able to bind to the anode, thereby decreasing the reaction sites available

(Song, 2002). The removal of such trace impurities requires complex and expensive feed processing which disproportionately increases the overall operating cost.

Recently, Kim and Chang (2009) proposed an alternative method of syngas conversion to electricity by a bioelectrochemical process, where a CO fermenter was connected to a microbial fuel cell (MFC). Subsequently, we, for the first time, demonstrated electricity generation in an MFC directly fed with CO or a mixture of CO and H_2 (Mehta et al., 2010). In an MFC, microorganisms are employed as a catalyst for electricity generation, which allows this contraption to be successfully operated on a wide range of substrates and varied operating conditions with high efficiency. (Choi, 2004; Demirbas, 2007; Jong et al., 2006; Logan, 2008; Mathis et al., 2008). Moreover, the utilization of microbial catalysts promises higher durability as the catalyst is self-regenerating. These inherent merits along with intrinsic resistance of the microbial catalysts to poisoning by fuel impurities such as sulfur, give MFCs an edge over other conventional processes, and suggest that MFCs could offer a robust and an efficient technology for CO/syngas conversion to electricity.

1.2 Problem Statement

We recently reported electricity production in an MFC directly fed CO/syngas (Mehta et al., 2010). Though the study clearly demonstrated that microbial population of an MFC are capable of utilizing CO/syngas as an electron donor for electricity production, the performance of the system in terms of volumetric power output, Coulombic efficiency and CO/syngas transformation efficiency was low. Efficient MFC operation on CO/syngas poses a number of engineering and microbiological challenges pertaining to poor solubility of the gaseous substrates in the liquid phase, understanding of the biochemical pathways involved

in electricity production, and selection of electrode materials and microbial catalysts resistant to poisoning by CO and sulfur compounds.

1.3 Objective

The primary objective of this PhD research was to enhance the overall performance of a CO/syngas-fed MFC in terms of its volumetric power output, Coulombic efficiency and gas conversion efficiency by improving the reactor design and operating conditions, and elucidate the microbial ecology and biochemical pathways involved in electricity production.

1.3.1 Specific Objectives

1) Demonstrate that the volumetric power output of a CO/syngas-fed MFC can be enhanced by improving CO/syngas gas to liquid transfer.
2) Analyse the microbial communities and comprehend the biochemical pathways involved in electricity production in a CO or syngas-fed MFC operated under mesophilic conditions.
3) Demonstrate electricity production and consequently CO/syngas dependant bioelectrochemical activity under thermophilic conditions.
4) Analyse the microbial communities and comprehend the biochemical pathways involved in electricity production in a CO/syngas-fed MFC operated under thermophilic conditions.
5) Develop a multi-anode/cathode MFC configuration to enhance the power output and Coulombic efficiency on syngas, and systematically investigate the impact of different electrode arrangements and operating temperatures on the system's performance.

CHAPTER 2

Review of Literature

2.1 Microbial Fuel Cells (MFC)

MFC represents a novel technological solution for electricity production from biomass. The technology exploits the ability of certain microorganisms such as *Geobacter sulfurreducens*, *Rhodoferax ferrireducens*, *Shewanella putrefaciens*, etc., that are capable of extra-cellular electron transfer to an insoluble electron acceptor such as an electrode. Such microorganisms are commonly referred to as anodophilic, exoelectrogens or electricigens and the process is referred to as electrogenesis or electricigenesis (Logan, 2008).

An MFC in its simplest form consists of two compartments, the anode and cathode compartment, which are often separated by a proton-exchange membrane or simply by a non-conductive synthetic separator. The compartments are connected by an external electrical circuit as shown in Figure 2.1 below.

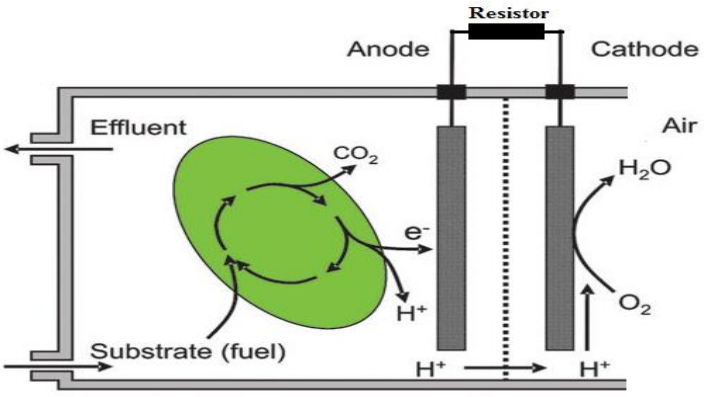

Figure 2.1 Schematic of a typical single chamber MFC (adapted from Logan 2008).

The anodic chamber houses the electricigenic microorganisms which are laid upon a convoluted, conductive and non-corrosive anode such as graphite brush, carbon felt, etc., forming a biofilm. This biofilm first catalyzes the oxidation of the fed fuel (electron donor), liberating electrons and protons and then transfer the electrons to the anode (Rinaldi et al., 2008). As an example, the oxidation of glucose in the anodic chamber is presented in equation 2.1 below:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \quad \text{.....} \quad (2.1)$$

While the electrons travel through an electrical load (device to be powered or a resistor in lab scale studies) and generate electricity until reaching the cathode, the corresponding protons migrate through the separator to the cathodic compartment to maintain the electrical neutrality. At the cathode, the protons combine with the electrons and an electron acceptor (catholyte) such as oxygen, to form H_2O through a reduction reaction as shown in equation 2.2 below:

$$6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O \quad \text{.....} \quad (2.2)$$

MFCs have been successfully operated on a wide range of substrates such as acetate, H_2, glucose, galactose, butyrate, starch, marine sediments, swine wastewater etc. (Pant et al., 2010). In principle, any bio-degradable material could be utilized as a fuel for electricity generation in an MFC. An ideal MFC can produce current while sustaining a steady voltage as long as a steady supply of substrate in maintained. The theoretical ideal voltage, E_{ideal} (V) attainable in an MFC can be thermodynamically predicted by the Nernst equation:

$$E_{ideal} = E^0 - \frac{RT}{nF}\ln(\prod) \quad \text{.....} \quad (2.3)$$

Where, E^0 is the standard cell potential (V), R is the universal gas constant (8.314 J $mol^{-1}K^{-1}$), T is the temperature (K), n is the number of electrons transferred in the reaction (dimensionless), F is the Faraday's constant (96,485 C mol^{-1}) and $\prod$ is the chemical activity of the products divided by those of the reactants (dimensionless).

In practice the actual voltage attainable in an MFC is less than the predicted voltage due to various irreversible losses or overpotentials. Activation, ohmic and mass transport losses are the three major irreversible losses that affect MFC performance (Rismani-Yazdi et al., 2008). Briefly, activation losses are due to the activation energy that must be overcome by the reacting species at each electrode and largely depends on the electrochemical properties of the deployed electrodes, current density of the anode, operating temperature and presence of electrochemical mediators. Hence, such voltage losses could be minimized by improving the electrode configuration and surface area, increasing the operating temperature and by utilizing microorganisms with high bio-electrochemical activity or enriching the electrode chambers with electro-mediating compounds (Rinaldi et al., 2008). Ohmic losses can be broadly ascribed to the electronic flow through the electrodes, the current collectors and the contact, and also to the resistance to the flow of ions through the electrolyte. Such losses could be reduced by improving the reactor design to reduce the distance between the electrodes, utilization of proton exchange membranes (if used) with low resistance and by increasing the electrolyte conductivity. Concentration losses represent the voltage losses due to the depletion of the reactants in the electrolyte near the electrodes and the accumulation of the reaction products. Improvement in the reactor configuration and operating parameters to reduce the concentration gradient minimizes such losses. The actual cell voltage, *E*cell (V) of an MFC can thus be determined by

subtracting the voltage losses in anodic and cathodic compartment and can be described by the following equation (Rinaldi et al., 2008):

$$E_{cell} = [E_{cathode} - |\eta_{act,c} + \eta_{conc,c}|] - [E_{anode} - |\eta_{act,a} + \eta_{conc,a}|] - \eta_{ohm} \quad \text{..... (2.4)}$$

Where, $E_{cathode}$ and E_{anode} represent the cathode and anode potentials (V), $\eta_{act,c}$ and $\eta_{act,a}$ represent the activation losses in the cathodic and anodic chamber, $\eta_{conc,c}$ and $\eta_{conc,a}$ represent the concentration losses in the cathodic and anodic chamber, and η_{ohm} represents the ohmic losses.

2.2 Calculations and procedures of reporting data

Power output and Coulombic efficiency

The overall performance of an MFC can be evaluated in many ways, but power output and Coulombic efficiency are the two parameters most commonly used in evaluating/reporting the efficiency of an MFC. Power output of a lab-scale MFC is calculated by measuring the voltage, E_{cell} across the load (R_{ext}) and current as:

$$P = \boldsymbol{I.\ E_{cell}} \quad \text{..... (2.5)}$$

Based on Ohms law, we can alternatively express power output as:

$$P = \frac{E_{cell}^2}{R_{ext}} \quad \text{..... (2.6)}$$

Power produced by an MFC is generally normalized to some characteristic reactor parameter to make it possible to compare the performance of different reactors. The choice of the parameter used for normalization usually depends on the targeted application/novelty of the system, as not all systems are optimized for power production (Logan, 2008). However, the electrode surface area (eq 2.7) and the total reactor volume

(eq 2.8) are the two most commonly utilized parameters for estimating the power density (W/m^2 or W/m^3).

$$P = \frac{E_{cell}^2}{A_{elect} R_{ext}} \quad \text{..... (2.7)}$$

where, A_{elect} is the anodic or cathodic surface area (m^2)

$$P = \frac{E_{cell}^2}{V.R_{ext}} \quad \text{..... (2.8)}$$

Where, V is the total reactor volume (m^3 or L)

Coulombic efficiency (CE) is defined as the ratio of total coulombs transferred to the anode from the fed substrate, to the theoretical maximum number of coulombs recoverable from the added substrate. In other words, CE presents the total number of electrons recovered as current versus the total recoverable electrons in the substrate (Logan, 2008). For a continuous fed system, CE can be estimated based on the average current generated under steady state conditions as shown below:

$$CE = \frac{I.\Delta t.M_{sub}}{F.n\,(M_{sub,in} - M_{sub,out})} 100\% \quad \text{..... (2.9)}$$

Where, I is the average measured current (A), Δt is the time interval during which the current was measured (s), M_{sub} is the molar mass of the fed substrate (g/mol), F is the faraday constant (C/mol-e^-), n is the number of electrons exchanged per mol of substrate (mol-e^-/mol), $M_{sub,in}$ is the amount of substrate (g) fed to the MFC during Δt, and $M_{sub,out}$ is the amount of substrate recovered (g) in the MFC effluent or off-gas during Δt.

Polarization and power density curves

Polarization and power density curves present a powerful tool for the analysis and characterization of fuel cells. A polarization curve represents the voltage as a function of

current and is obtained by inducing a periodic decrease of the external load (R_{ext}) and recording the corresponding voltage. The current is then calculated using ohms law. By plotting voltage versus current or current density (normalizing by electrode surface area or reactor volume), a polarization curve as shown in Fig 2.2 (A) is obtained. The curve illustrates how well the MFC maintains a voltage as a function of the current production. Correspondingly, a power curve as shown in Fig 2.2 (B), illustrates power or power density as a function of current (density).

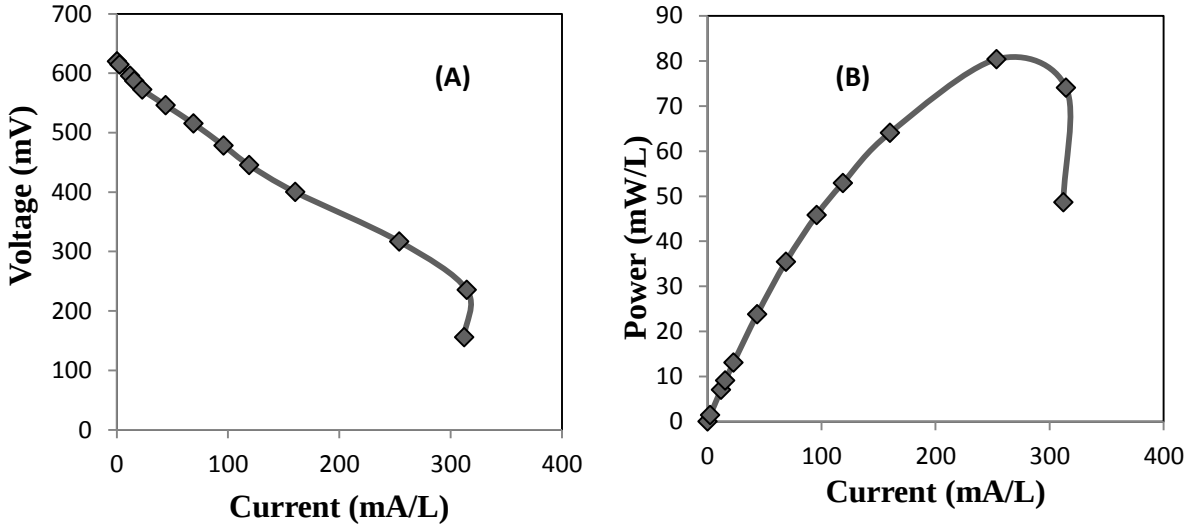

Figure 2.2 Example of polarization (A) and power curve (B) of a microbial fuel cell, where power and current density were obtained by normalizing to the total reactor volume (L).

Polarization curve is also utilized for the estimation of MFC internal resistance (R_{int}). The internal resistance, including activation, ohmic and concentration losses is an important parameter that governs the performance of an MFC. R_{int} can be quickly estimated by the polarization slope method, where the slope of a voltage versus current plot (polarization curve) represents the internal resistance of the MFC.

2.3 Electricity production from CO and syngas in an MFC

The possibility of electricity production from CO and syngas in an MFC has been demonstrated only recently. Kim and Chang (2009), demonstrated electricity production from CO in a two-stage reactor system as shown in Fig. 2.3, in which CO was first microbiologically converted to fermentation products (dominantly acetate), which were subsequently fed to an MFC seeded with an anaerobic sludge. A maximum power output of 1.6 mW L^{-1} (normalized to the total reactor volume) and Coulombic efficiency of ~ 5 % were reported.

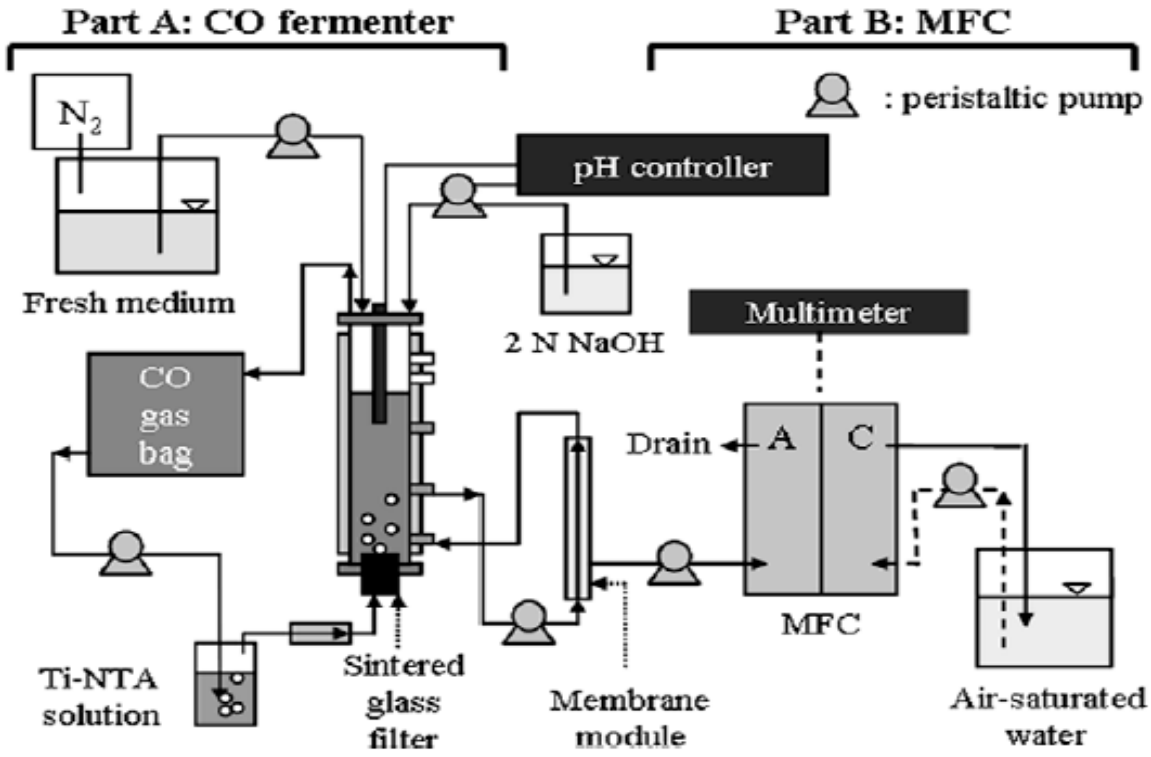

Figure 2.3 Schematic of the two-stage process utilized in the study of Kim and Chang (2009), for electricity production from carbon monoxide.

Instead of a two-stage process, we demonstrated electricity generation in an MFC directly fed with CO/syngas (Mehta et al., 2010). The maximum volumetric power output and Coulombic efficiency (CE) of the system were 6.4 mWL^{-1} and 8.7 %, respectively. Though the overall performance of the MFC directly fed with CO/syngas was only marginally better than the two-stage process utilized in the study of Kim and Chang (2009), it clearly demonstrated that the microbial communities of an MFC could utilize CO/syngas as an electron donor for electricity generation. The low performance of the MFC on CO/syngas was attributed to the inefficient system design and gas delivery. The utilization of a sparger system for gas delivery (Fig. 2.4) not only required the doubling of the reactor volume which invariably resulted in low power density, but also the inefficient gas-liquid mass transfer required much higher CO/syngas feed in rates to reach the desired dissolved CO levels; resulting in considerable gas losses and low CO transformation efficiency. This suggested that the performance of an MFC on CO/syngas could be enhanced by adoption of an efficient gas-liquid mass transfer mechanism and reactor design optimization.

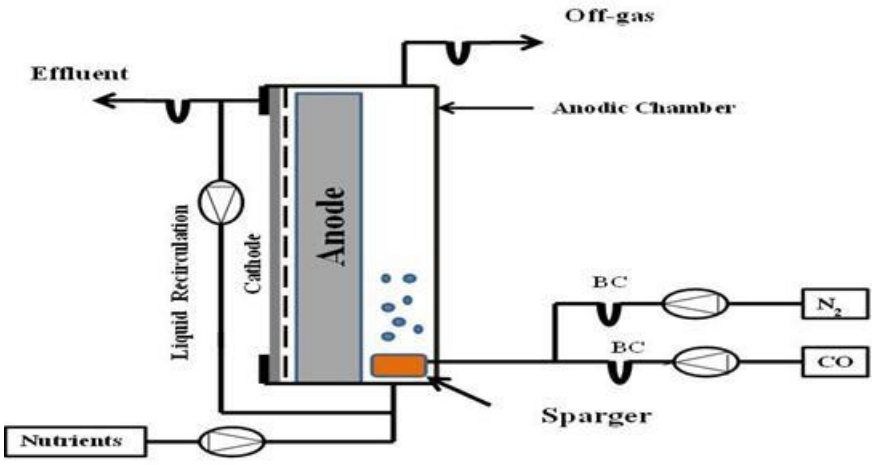

Figure 2.4 Schematic of the experimental set-up utilized in the study of Mehta et al. (2010), for electricity generation from carbon monoxide/syngas.

Based on the analysis of metabolic products, we concluded that the production of electricity from CO or syngas in an MFC proceeds through a multi-step biotransformation process (Mehta et al., 2010). Several concurrent pathways, as shown in Figure 2.5, were hypothesized: One pathway involved CO transformation to acetate by acetogenic carboxidotrophic (CO oxidizing) microorganisms followed by oxidation of acetate by CO tolerant electricigenic microorganisms (pathway 1-4 in Fig. 2.5). This pathway was hypothesized to be the foremost responsible for electricity generation. Acetate injection in the MFC always led to a dramatic increase in power production. In addition to acetate, other degradation products such as hydrogen were also found in the off-gas samples during MFC operation solely on CO, which indicated the presence of hydrogenogenic carboxydotrophic microorganisms. It was hypothesized, that H_2 is also utilized for electricity production by the electricigenic microorganisms (pathway 3-5 in Fig. 2.5). The importance of this pathway might be increased in the syngas-fed MFC where a significant amount of hydrogen is present. Notably, the ability of the electricigenic microorganisms to utilize H_2 as an electron donor has been documented (Bond & Lovley, 2003). The presence of acetate when the MFC was fed only with hydrogen also indicated the presence of homoacetogenic microorganisms. Based on this observation a pathway of electricity production through H_2 and acetate followed by acetate conversion to electricity was also suggested (pathway 2-3-4 in Fig. 2.5). The work of Mehta et al (2010) also raised a possibility of another pathway, which involved direct electron transfer to the anode by the metal reducing carboxidotrophic bacteria (pathway 5 in Fig. 2.5). Microorganisms capable of utilizing CO as an electron donor for metal reduction such as *Thermosinus carboxydivorans*, *Carboxydothermus ferrireducens* etc. have been isolated recently (Sokolova et al., 2004).

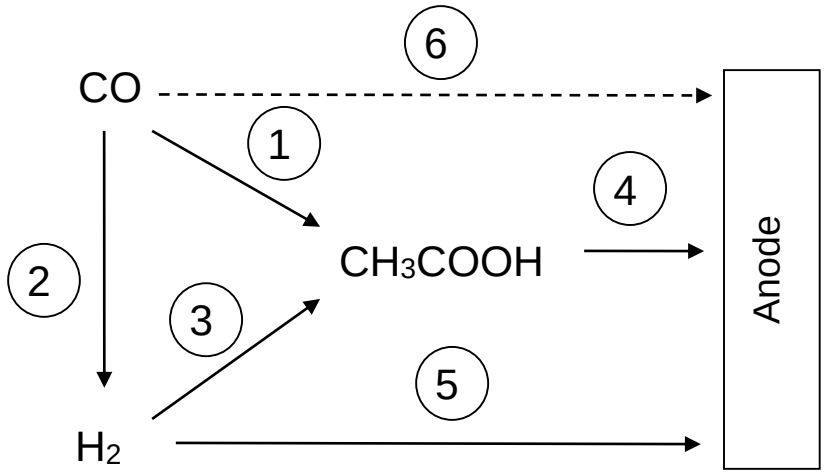

Figure 2.5 Proposed pathways of electricity production from CO and syngas in an MFC (Mehta et al., 2010). Notations: 1 – CO conversion to acetate by acetogenic carboxidotrophs, 2 – CO conversion to H_2 by hydrogenogenic carboxidotrophs, 3 – H_2 conversion to acetate by homoacetogens; 4, 5 – acetate and H_2 consumption by electricigenic microorganisms, 6 – CO consumption by electricigenic carboxidotrophs (hypothesized).

Based on the experimental observations from the studies of Kim and Chang (2009) and Mehta et al. (2010), it is evident that the electricity production from syngas in an MFC poses a number of engineering and microbiological challenges pertaining to gas transfer limitations, selection and enrichment of microorganisms capable of efficient syngas transformation to electricity, and selection of cathodic catalysts resistant to poisoning by CO and sulfur compounds. The subsequent sections of this chapter review the microbial communities and reactor designs suitable for MFC operation on CO/syngas.

2.4 Microbial community of a CO and syngas-fed MFC

This section discusses microbial communities that could be realized in an MFC for efficient CO/syngas transformation to electricity based on the biochemical pathways discussed in the previous section and as illustrated in Figure 2.5.

2.4.1 Syngas conversion to electricity by acetogenic and electricigenic microorganisms ($CO/H_2 \rightarrow$ Acetate $\rightarrow$ e^-)

Mesophilic acetogenesis and electricigenesis

Acetogens have the ability of utilizing wide range of substrates for metabolic needs (Drake & Daniel, 2004). *Clostridium carboxidivorans* (ATCC BAA 624), *Clostridium autoethananogenum* (DSM 10061), *Peptostreptococcus productus* (ATCC 35244), *Eubacterium limosum* (ATCC 10825) *and Acetobacterium woodii* (DSM 1030) are some of the mesophilic bacterial acetogens capable of using CO as their sole source of energy while forming acetate and CO_2 (Genthner & Bryant, 1987; Henstra et al., 2007; Lorowitz & Bryant, 1984). This biotransformation can be presented by the following stoichiometric equation:

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \quad \text{.....} \quad (2.10)$$

P. productus isolated from an anaerobic sewage digester was the first acetogenic anaerobic coccus observed to utilize CO as an energy source. The doubling time with 90% CO was 1.15 h at an optimum temperature of 30 °C (Lorowitz & Bryant, 1984). Similarly, *Eubacterium limosum* can utilize CO to form acetate and CO_2. The generation time of *E. limosum* on CO, within the temperature range of 38-39 °C, was reported to be 7 h. *A.*

woodii grew on CO at 30 °C without requiring any adaptation period with a generation time of 13 h.

Acetate is also formed by certain bacteria by acetogenic hydrogenation where H_2 and CO_2 are converted to acetate according to the following stoichiometric equation (step 3 in Fig. 2.5):

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad (2.11)$$

This ability under mesophilic conditions has been demonstrated by *Clostridium aceticum* (DSM 1496), *Acetobacterium wieringae* (DSM 1911), *Ruminococcus hydrogenotrophicus* (DSM 10507), etc. (Bernalier et al., 1996; Braun et al., 1981). *C. aceticum* is an obligate anaerobe, which grows chemolithotrophically on H_2 and CO_2 forming acetate. The optimum temperature and pH for its growth was reported to be 30 °C and 8.3, respectively, with a doubling time of 25 h (Braun et al., 1981). Similarly, *A. wieringae* coupled H_2 and CO_2 for acetate formation. The doubling time was less than 10 h at the optimal growth temperature and pH of 30 °C and 7.6 respectively (Braun & Gottschalk, 1982). *R. hydrogenotrophicus* is a strict anaerobe that grew autotrophically on H_2 and CO_2 forming acetate (Bernalier et al., 1996).

For electricity generation under mesophilic conditions, the above mentioned bacteria could be used in co-culture with electricigenic microorganisms capable of using acetate as an electron donor such as *Geobacter sulfurreducens* (ATCC 51573) (Bond & Lovley, 2003), leading to a syntrophic relationship with the acetogens. The following stoichiometric equation can be used to describe the corresponding acetate transformation reaction (Logan, 2008):

$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+$ ..... (2.12)

Equations (2.10-2.12) imply a yield of 2 e^-/mol either on CO or on H_2 for a syngas-fed MFC. A detailed review of electricigenic microorganisms can be found elsewhere (Logan, 2009; Logan & Regan, 2006; Lovley, 2008). Interestingly, genome sequencing of *G. sulfurreducens*, often found in mixed microbial populations of MFCs, revealed the presence of carbon monoxide dehydrogenase, which is known to catalyze the reaction of CO conversion to CO_2 and H_2 (Methe et al., 2003). Although growth of *G. sulfurreducens* on CO has not been reported, it can be suggested that this strain can be at least tolerant to the presence of CO, thus growing in a co-culture with carboxydotrophic strains producing acetate.

Thermophilic acetogenesis and electricigenesis

The growth of acetogens on CO and electricity generation through the acetate pathway can also be anticipated under thermophilic conditions. Many acetogens capable of growth on CO under thermophilic conditions have been reported. Savage et al. (1987) reported the ability of CO-dependent chemolithotrophic acetogenesis and growth by *Moorella thermoautotrophicum* (ATCC 33924) (earlier *Clostridium thermoautotrophicum*), with supplemental CO_2 required for efficient growth on CO. The CO/CO_2 ratio of 2:4 yielded optimal doubling times at a temperature of 58 °C. This microorganism has the ability to grow autotrophically and heterotrophically using various electron donors and acceptors (Savage et al., 1987; Sokolova et al., 2009). *Clostridium thermoaceticum* (ATC35608), also demonstrated CO dependent growth and acetogenesis under chemolithotrophic conditions with a doubling time of 10 h at a temperature of 55 °C (Daniel et al., 1990). The recently isolated thermophilic bacterium *Moorella perchloratireducens* (ATCC BAA

1531), which is closely related to the above mentioned bacterial species, resorted to acetogenesis in the absence of perchlorate (Balk et al., 2008).

Acetogenic hydrogenation (Eq. 2.11) has also been observed in thermophiles. *C. thermoaceticum*, capable of growth on CO, grew chemolithotrophically on H_2 and CO_2 forming acetate. The doubling time was 18 h at a temperature of 55 °C (Kerby & Zeikus, 1983). A similar growth physiology was also observed in *Acetogenium kivui* (ATCC 33488) (Daniel et al., 1990). This thermophilic anaerobic bacterium formed acetate by chemolithotrophic growth on H_2 and CO_2 with a doubling time of 2 h, at a temperature of 66 °C and pH of 6.8.

For generation of electricity in an MFC the above mentioned thermophilic bacterial species have to be co-cultured with thermophilic electricigenic microorganisms capable of using acetate as an electron donor. Although the microorganisms studied for generation of electricity in an MFC are predominantly mesophilic (e.g. *G. sulfurreducens* or *G. metallireducens*), successful MFC operation under thermophilic conditions has been demonstrated. Choi (2004), used thermophilic bacteria *Bacillus licheniformis* and *Bacillus thermoglucosidasius* (with a redox mediator) for electricity generation. The best efficiency was achieved within the temperature range of 50–60 °C. Mediatorless MFC operation under thermophilic conditions was reported by Jong et al., (2006). The MFC was inoculated with anaerobic digester effluent and fed with sodium acetate. The maximum power density was achieved during MFC operation at 55 °C. Based on the 16S rRNA analysis, only 13 different patterns of anodic bacterial populations were observed, of which 5 patterns showed the highest homology to an uncultured clone E4, which was initially identified as a member of a thermophilic microbial community in a lab scale methanol-fed

anaerobic digester. Seven patterns were related to genus *Coprothermobacter* and one pattern was related to *Thermodesulfovibrio* spp.

In the study by Mathis et al. (2008), thermophilic bacteria selected from sediment MFC were used to colonize the anode of an acetate and cellulose-fed MFCs. Cloning and sequencing of the biofilm formed at the anode of the acetate fed MFC showed the presence of *Deferribacters* and *Fermicutes.* Interestingly, 48 clones (out of 64) of *Fermicutes* had RFLP patterns and sequences (99%) most similar to that of *Thermincola carboxydophila*, a hydrogenogenic CO oxidizing thermophilic microorganism (Mathis et al., 2008). *Firmicutes* spp. were also identified during thermophilic MFC operation by Wringhton et al. (2008). This study provided a detailed analysis of microbial community dynamics in an acetate fed MFC inoculated with sludge collected from a thermophilic anaerobic digester. The dominant members of the electricity producing community were identified using clone library analysis. The results showed the dominance of *Firmicutes* spp. (80 % of the clone library sequences). Within *Firmicutes,* sequences belonging to *Thermicanus, Alicyclobacillus and Thermincola* were identified representing 27%, 25% and 22 % of the total clones, respectively. The study was well complemented by the demonstration of electricity production in an MFC inoculated with a pure strain of *Thermincola sp.* Strain JR, representing direct anode reduction by a member of *Fermicutes* phylum (Wrighton et al., 2008).

As electricity generation has never been an evolutionary pressure per se, but rather the capacity for electron transfer to natural extracellular electron acceptors, it is likely that the ability of the microorganism to produce electricity is closely correlated to their capacity to transfer electrons onto extracellular acceptors, such as Fe(III) and Mn(IV) oxides and

humic substances (Logan, 2009; Lovley, 2006; Lovley et al., 2004). Hence, the prospect for electricigenic microorganisms could include any microorganism capable of extracellular electron transfer, even if their capacity for electricity generation has not yet been experimentally evidenced (Lovley et al., 2004). To this respect, hyper-thermophiles *Ferroglobus placidus* (DSM 10642) and *Geoglobus ahangari* (ATCC BAA 425) were reported to grow at 85 °C by coupling acetate oxidation to Fe(III) reduction (Tor et al., 2001). Also, *Deferribacter thermophilus* (DSM 14813) isolated from a petroleum reservoir (UK) was able to grow by the reduction of Fe(III) and Mn(IV) and nitrate in the presence of acetate, yeast extract, peptone, and other carbon sources in the temperature range of 50–65 °C (Greene et al., 1997). Kashefi et al. (2003) reported the isolation of a bacterial strain belonging to the *Geobacteraceae* family exhibiting thermophilic growth. The bacterium, *Geothermobacter ehrlichii* (DSM 15274) isolated from a hydrothermal vent coupled acetate oxidation to Fe(III) reduction, with an optimum growth temperature of 55 °C. This strain is the first member in the *Geobacteraceae* family reported to be capable of thermophilic growth. Fe(III) reduction coupled to acetate oxidation has also been demonstrated by the bacterium *Thermincola ferriacetica* (DSM 14005) (Zavarzina et al., 2007). Overall, a broad range of thermophilic electricigenic microorganisms might be capable of forming a syntrophic consortium with thermophilic carboxidotrophic microorganisms for efficient operation of a syngas-fed MFC.

2.4.2 Syngas conversion to electricity by hydrogenogenic and electricigenic microorganisms ($CO \rightarrow H_2 \rightarrow e^-$)

Biological water gas shift reaction and electricigenesis at mesophilic temperatures

This pathway leads to CO conversion to H_2 through the biological water gas shift reaction (BWGSR) followed by H_2 utilization as an electron donor by electricigenic bacteria. Indeed, the ability of CO dependent H_2 production by mesophilic bacteria such as *Rubrivivax gelatinosus* (Maness et al., 2005), *Rhodospirilum rubrum* (Singer et al., 2006), *Rhodopseudomonas palustris* P4, and *Citrobacter* sp Y19 (Henstra et al., 2007) has been reported. *R. gelatinosus* uses CO as the sole carbon source (with a doubling time of 2 days), leading to the generation of H_2 according to the following stoichiometric equation, which describes the water-gas shift reaction (Maness et al., 2005) :

$$CO + H_2O \rightarrow H_2 + CO_2 \quad \text{(2.13)}$$

Similarly, the exposure of *R. rubrum* to CO leads to H_2 production according to Eq. (2.13) due to the stimulation of a CO oxidizing – H_2 evolving enzymatic system (Singer et al., 2006). In a syngas-fed MFC this pathway is expected to maximize the Coulombic efficiency of syngas transformation, as compared to H_2 utilization through acetate formation. To conclude, a mixed culture of electricigenic and mesophilic carboxydotrophic hydrogenogenic bacteria would allow for CO conversion to H_2 and the subsequent use of the H_2 produced from CO and H_2 present in syngas for electricity generation.

Biological water gas shift reaction and electricigenesis at thermophilic temperatures

The sequence of bioreactions described above is also expected to proceed under the thermophilic conditions since thermophilic oxidation of CO leading to H_2 evolution is a widespread trait found among several recently isolated carboxydotrophic microorganisms.

Carboxydotrophic microorganisms such as *Thermolithobacter carboxydivorans* (DSM 7242), *Carboxydothermus hydrogenoformans* (DSM 6008), *T. carboxydophila* (DSM 17129), *Carboxydocella thermoautotrophica* (DSM 12326), *Themolithobacter carboxydivorans*, and *Carboxydibrachium pacificum* (ATCC BAA 271) produce H_2 from CO oxidation under thermophilic growth conditions (Sokolova et al., 2007; Sokolova et al., 2009; Sokolova et al., 2005; Sokolova et al., 2002). A number of carboxydotrophic microorganisms capable of hydrogenogenic activity have been isolated from marine hydrothermal vents (Henstra et al., 2007; Sokolova et al., 2009). *C. pacificum*, isolated from a submarine hot vent grew chemolithotrophically on CO producing equimolar quantities of H_2 and CO_2. Its growth was observed between 50 and 80 °C with an optimum temperature of 70 °C (Sokolova et al., 2002). Likewise, *C. thermautotrophica*, a thermophilic CO utilizing bacterium isolated from a terrestrial hot vent on the Kamchatka Peninsula (Russia) produced H_2 and CO_2 with a generation time of 1.1 h at a temperature of 58 °C and pH 7. *Carboxydocella sporoproducens* (DSM 16521), also isolated from hot springs of karymshoe Lake, Kamchatka Peninsula (Russia) grows chemolithotrophically on CO (doubling time of 1 h) producing equimolar quantities of CO_2 and H_2. The temperature and optimum pH were observed to be 60 °C and 6.8, respectively (Slepova et al., 2006). Sokolova et al. (2005) reported the isolation of the alkali-tolerant carboxydotrophic hydrogenic bacterium, *T. carboxydophila*, from a hot spring of the Baikal Lake region, Russia. CO was found to be the sole source of energy for this bacterium. For lithotrophic growth of *T. carboxydiphila*, acetate or yeast extract were required, but these substrates did not support growth in the absence of CO. Neither acetate nor methanol formation was detected during its growth on CO.

Similar to the mesophilic co-culture, a co-culture of thermophilic hydrogenogenic carboxydotrophic microorganisms with H_2 utilizing thermophilic electricigenic microorganisms such as *D. thermophilus* or *Pyrobaculum islandicum* (DSM 4184) could be used for electricity generation in a syngas-fed MFC. *D. thermophilus* was able to grow by the reduction of Fe(III), Mn(IV) and nitrate in the presence of H_2. Similarly, *P. islandicum* is able to reduce Fe(III) and Mn(IV) with H_2 as an electron donor (Kashefi & Lovley, 2000). *Thermolithobacter ferrireducens* (ATCC 700985) reduces Fe(III), anthraquinone-2,6-disulfonate(AQDS), thiosulfate and fumarate with H_2 serving as the electron donor in a temperature range of 50–75 °C (Sokolova et al., 2007).

2.4.3 Direct Conversion of CO to electricity by axenic cultures of Fe(III) and sulfate reducing thermophiles

While direct conversion of H_2 to electricity has been experimentally demonstrated (Bond & Lovley, 2003), electricigenic bacteria growth on CO has not yet been demonstrated. Nevertheless, Sokolova et al. (2004) reported the isolation of a new anaerobic facultative carboxydotrophic bacterium from a hot spring at Norris basin (Yellowstone National Park, US). The bacterium, *Thermosinus carboxydivorans* (DSM 14886), grew at temperatures between 40 and 68 °C (with an optimum at 60 °C) at neutrophilic conditions. The bacterium could utilize CO as its sole energy source with a doubling time of 1.15 h leading to the formation of H_2 and CO_2 in equimolar quantitites. Fe(III) was also reduced during its growth on sucrose and lactose. The species was the first metal reducing carboxydotrophic bacterium to be reported (Sokolova et al., 2004). Similarly, *Carboxydothermus ferrireducens* (DSM 11255) also isolated from Yellowstone National Park (Henstra & Stams, 2004; Sokolova et al., 2009), has the ability to use CO as

an electron donor for AQDS and fumarate reduction. Fumarate, AQDS, ferric iron and thiosulfate could serve as electron acceptors during its growth on glycerol and H_2. Hydrogen or acetate production was not observed during its growth on CO. In contrast, *Carboxydothermus siderophilus* (DSM 21278) isolated from hot spring of Geyser Valley (Kamchatka Peninsula, Russia), produced H_2 and CO_2 along with Fe(III) and AQDS reduction during its growth on CO (Slepova et al., 2009). However, the doubling time for *C. siderophilus* (9.3 h) on CO was much longer than that of *T. carboxydivorans* (1.15 h). Balk et al. (2008) reported the isolation of *Moorella perchloratireducens*, the thermophilic gram positive bacterium with the ability to use perchlorate as a terminal electron acceptor. This strain was able to use CO, methanol, pyruvate, glucose, fructose, mannose, xylose, pectin and cellobiose for its growth.

T. ferriacetica (DSM 14005), isolated from ferric deposits of a terrestrial hydrothermal spring (Kunashir Island, Russia), is a thermophilic facultative chemolithoautotrophic anaerobic bacterium. It was able to utilize H_2 and acetate as energy sources, with Fe(III) serving as the electron acceptor. Also, it was able to grow in an atmosphere of 100% CO (as the sole energy source), leading to the formation of H_2 and CO_2. However, it required 0.2 g/L of acetate as its carbon source during its growth on CO (Zavarzina et al., 2007). *C. hydrogenoformans*, a close relative of *T. ferrireducens*, oxidized CO and H_2 using AQDS as an electron acceptor. CO_2 and H_2 were formed during its growth on CO.

It should be noted that most of the metal-reducing carboxydotrophic organisms are thermophiles. High temperatures may be more favorable as less cooling of syngas would be required (Henstra et al., 2007). Although MFC operation at thermophilic temperatures is expected to have a detrimental effect on the CO solubility in the anodic liquid, this is

counteracted by the increase in the mass transfer rate with increasing temperature (Drew, 1981). Elevated temperatures would also lead to reduced O_2 solubility, which is beneficial considering the sensitivity of carboxydotrophs to O_2 (Davidova et al., 1994).

2.5 Design considerations for electricity generation from CO/syngas in an MFC

Apart from the biological system, the efficient generation of electricity in a CO/syngas-fed MFC would also require the optimization of the MFC design to counter the two major challenges posed by a gaseous substrate such as syngas: gas to liquid mass transfer limitations, and the poisoning of the cathode by CO and other toxic gases.

2.5.1 Membrane systems for improved mass transfer efficiency

For sparingly soluble gases such as CO and H_2, the primary resistance to gas transport is in the liquid film at the gas-liquid interface. Although the conventional continuous stirred tank reactors could be used, the high impeller speeds require a high power input (Henstra et al., 2007; Hickey et al., 2008) and leads to biofilm shearing, causing a decrease in the growth of shear-sensitive microorganisms (Munasinghe & Khanal, 2010).

Bubble-free gas transfer to liquid can be accomplished by the selection of a membrane system with a high selectivity for the gaseous substrate. The membrane systems offer an efficient and a relatively inexpensive method for gas–liquid mass transfer (Scott and Hughes 1996). A wide range of micro-porous and non-porous membranes are commercially available and can be selected based on the substrate and process conditions. Depending upon the gas and process operational characteristics, a dense polymer or a micro-porous membrane could be employed. In a dense polymer membrane, the gas is absorbed in the polymer at the high pressure end and is carried to the liquid (lower pressure

side) by diffusion across the membrane. In contrast, the gas in a micro-porous system is transported to the liquid through the pore system rather than through the polymer. Although the gas pressure is maintained below the bubble point, bubbles might be formed at the surface of the membrane and kept attached to the membrane due to surface tension. Dense polymer membranes offer an advantage over micro-porous membranes as they can be operated at high pressures, which increases the concentration gradient and hence the mass transfer rate (Ahmed & Semmens, 1992; Côté et al., 1989).

In particular, silicone membranes are dense polymer membranes which offer the advantage of high mechanical strength, flexibility and stability under high temperature and pressures. They have been reported to be ideal for membrane based bubble-less aeration without vigorous mixing where a conventional system is unable to meet the O_2 requirements of a high rate system (Côté et al., 1989). They also find wide applicability in modified atmosphere storage for fruits and vegetables because of efficient regulation of the gas levels in the storage chamber due to their selective gas permeation properties (Gariépy et al., 1994). Though silicone membranes have not yet been extensively studied for CO transfer from gas to liquid, they have been reported to have much higher diffusion rates for gases in comparison to other hydro-carbon polymers (Robb, 1968). Therefore, it could be suggested that silicone membranes might offer an efficient membrane based mass transfer system for CO delivery. Depending upon the type of membrane selected, the membrane system offers the flexibility of being folded into different geometries (e.g. tubular or flat membrane) to increase the surface to volume ratio. Such membranes could be easily incorporated in a conventional MFC design as shown in Figure 2.6. A gas compartment containing syngas can be attached to the anodic compartment with the selected membrane

system acting as a wall between the anodic liquid and gas phase. The replacement of the sparger system utilized in the study of Mehta et al. (2010) with a membrane system would offer the added vantage of a more compact design. Indeed, the sparger used in the experiments carried out by Mehta et al. (2010) required a 50% increase of the anodic compartment. Thus, a membrane-based MFC is expected to have a higher volumetric efficiency. The details of different membrane systems made up of polyvinyl fluoride, polyethylene, polyvinyl chloride and other polymeric materials can be found elsewhere (Hickey et al., 2008; Scott & Hughes, 1996).

Apart from the membrane systems discussed above, other promising alternatives to conventional stirred tank reactors for increased gas-liquid mass transfer include monolith packing and columnar reactors. Monolith packing consists of a number of narrow, straight and parallel flow channels with a large open frontal area which allows for a low flow resistance, leading to low pressure drops and low energy losses. High volumetric mass transfer rates of ~ 1 s^{-1} and a reduction in power needed of up to 50 – 80% as compared to conventional reactors makes monolith reactors an economically viable option (Hickey et al., 2008; Munasinghe & Khanal, 2010). Similarly, columnar reactors such as bubble column, trickle bed and airlift reactors offer the advantage of a high gas – liquid mass transfer rate with low operational and maintenance costs. K_La values within the range of 18–860 h^{-1} have been reported for such reactors and can be found well summarized elsewhere (Bredwell et al., 1999; Charpentier, 1981; Munasinghe & Khanal, 2010). Various reactor design improvements such as the low frequency vibration of liquid phase in a bubble column reactor, the addition of static mixers, baffles, perforated plates, jet loop and forced circulation loop in internal and external loop airlift reactors promises further

increase in the gas–liquid mass transfer efficiency (Chisti et al., 1990; Ellenberger & Krishna, 2003; Fadavi & Chisti, 2005; Gavrilescu et al., 1997; Krichnavaruk & Pavasant, 2002; Ugwu & Ogbonna, 2002; Vorapongsathorn et al., 2001).

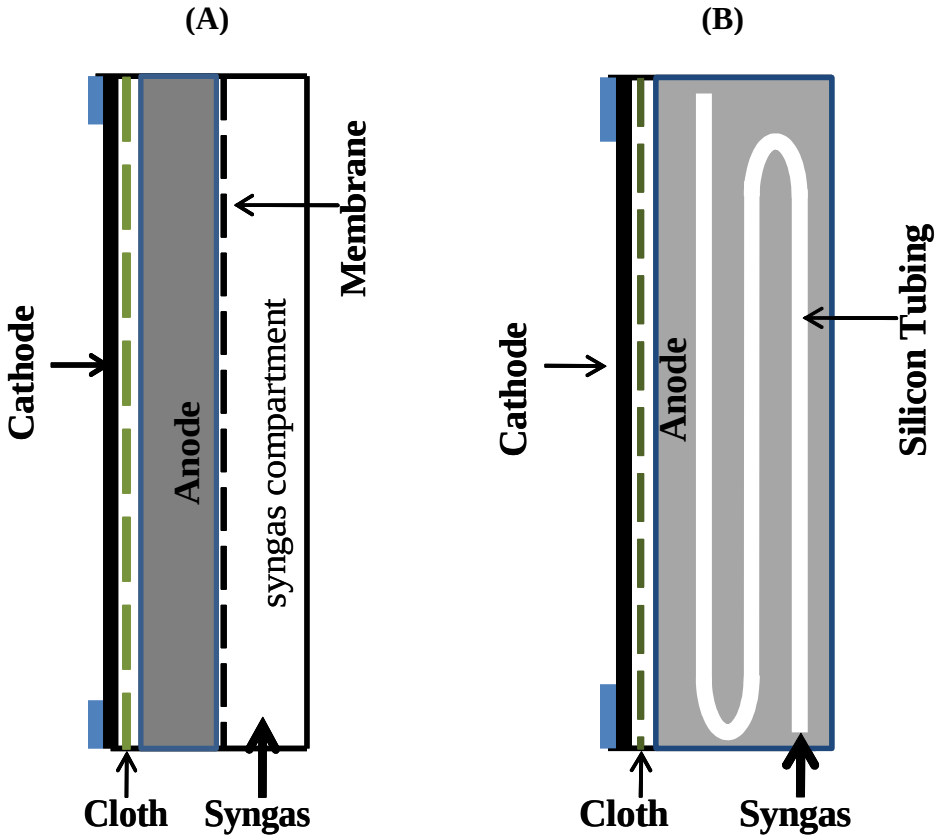

Figure 2.6 Proposed design of an MFC with (A) a flat membrane system and (B) microporous tubes embedded in the anode.

With an efficient gas to liquid mass transfer process in place, further improvements in MFC design/configuration to enhance power output (density) and Coulombic efficiency on CO/syngas can be realized. An efficient way of enhancing system performance on CO/syngas could be by the adoption of a multi-anode/cathode design. As the name suggests, a multi-anode/cathode design incorporates multiple electrodes into a single unit,

allowing for increased anodic and cathodic specific area (electrode area/reactor volume). On CO/syngas, increased anodic specific area may allow for increased density of carboxydotrophic and homo-acetogenic microorganisms and correspondingly high CO/syngas transformation efficiency, which when matched with increased electricigenic and cathodic activity may result in high volumetric power output and Coulombic efficiency. Considerable improvement in power outputs and Coulombic efficiency on waste water and other liquid substrates with the adoption of a multi-anode/cathode MFC configuration has been reported recently (Jiang et al., 2011; Jiang et al., 2010). The design for a CO/syngas-fed multi-anode/cathode MFC is provided is Figure 2.7.

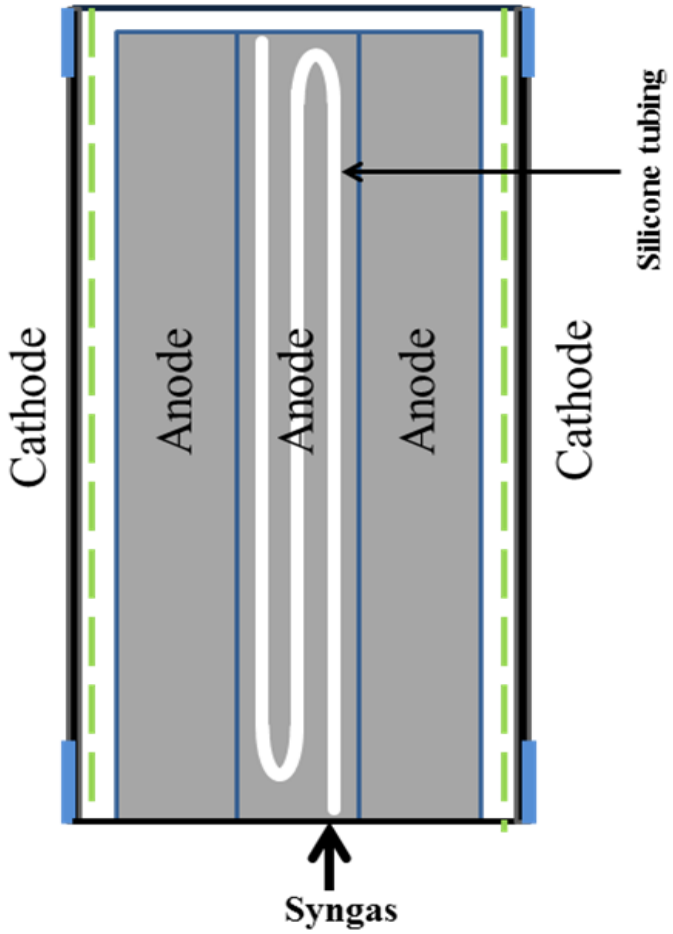

Figure 2.7 Proposed design of a syngas-fed multi-anode/cathode MFC

2.5.2 Non-noble catalysts for improved cathode stability

The development of an efficient MFC system for the generation of electricity from CO/syngas requires a CO-tolerant cathode. The most extensively used cathode material in a conventional MFC consists of a carbon paper with a Pt/C catalyst (Logan, 2008). Even though the Pt-based cathode demonstrates high electrochemical activity, the use of Pt is undesirable due to high costs and easy inhibition by CO (Herrmann et al., 2009; Logan, 2008). Even at small concentrations, CO can fully cover the Pt surface, thereby reducing the reaction site. CO is easily able to absorb to Pt due to the negative free energy of adsorption (Baschuk & Li, 2001). This phenomenon also poses a challenge for other conventional fuel cells such as direct methanol fuel cells and proton exchange membrane fuel cells (PEMFC), where even trace amounts of CO present as fuel impurity can seriously dampen the cells performance due to the relatively high loading of Pt at the anode and cathode (Baschuk & Li, 2001; Ormerod, 2003; Song, 2002; Steele & Heinzel, 2001).

Jasinski (1964) was the first to report that transition metal porphyrines and phthalocyanines demonstrated electrochemical activity towards oxygen reduction reaction and therefore can be used as cathode catalyst in fuel cells. Studies testing less expensive porphyrines and phthalocyanines as cathode catalyst in MFC such as Fe(II) phthalocyanine (FePc), and Fe(III) tetramethoxyphenyl porphyrin (FeTMPP), and Co tetramethoxyphenylporphyrin (CoTMPP) showed that these non-noble metal cathode catalysts can generate power equal to that obtained for Pt based carbon cathodes (Birry et al., 2010; HaoYu et al., 2009; Harnisch et al., 2009; Zhao et al., 2005). The use of CoTMPP as a cathode catalyst was also demonstrated by Cheng et al. (2005) where the

performance of a CoTMPP air cathode was tested in a single chamber MFC. A maximum power density of 369 ± 8 mW/m^2 was obtained using CoTMPP/C catalyst, which was only 12% lower than that obtained with a Pt/C catalyst (Cheng et al., 2005). When Fe loading was optimized for FePc-based cathodes, a similar power output was obtained in MFCs operated with FePc cathodes containing 0.01-016 mg Fe/cm^2 and 0.5 mg Pt/cm^2 (Birry *et al.* 2010).

In the study conducted by Mehta et al. (2010), a CoTMPP cathode was used for generation of electricity from CO with a cobalt (Co) load of 0.5 mg cm^{-2}. This was the first time that a CoTMPP cathode was used in an MFC operated on CO. In another study, we also tested pyrolised CoTMPP, FeTMPP and CoTMPP/FeTMPP cathode catalysts on acetate and CO (Neburchilov et al., 2011). An MFC operation on CO showed the best performance with the CoTMPP/FeTMPP/C cathode catalyst. Considering the high cost of Pt based cathodes and plausible decrease in activity with time, the use of CoTMPP/FeTMPP/C or FePc cathodes is a step forward in increasing the efficiency of CO operated MFCs.

2.6 Perspectives

Increasing energy demands, dwindling fossil fuel reserves, and environmental and health concerns have forced us to look for clean and sustainable sources of energy. If produced from biomass, syngas represents a renewable source of energy. An MFC represents a novel technological solution for direct electricity production from syngas. Significant improvements in design and operation have increased the power density and applicability of MFC systems. Power densities of up to 1 kW/m^3 under optimal operating conditions have been achieved (Logan, 2010). The conversion of CO/syngas to electricity

in an MFC, though at a very early experimental stage, could offer some major advantages such as high conversion efficiency, operation at temperatures in a range of 30-70°C, low maintenance requirements and operating costs, and most importantly resistance to CO poisoning. With the performance of a CO/syngas-fed MFC with a mixed culture as demonstrated recently, a detailed study of CO/syngas-operated MFC is of interest. This study should include the improvement of the system design to enhance the CO/syngas gas to liquid mass transfer, and possibly the development of stackable multi-anode/cathode MFC configuration capable of efficient operation on gaseous substrates such as CO and H_2. Given, that electricity generation in an MFC is governed by biological activity; characterization of the microbial communities and CO/syngas transformation pathways in a mixed culture under mesophilic and thermophilic conditions is of equal importance.

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Connecting text

Following the review of literature, it is evident that one of the major engineering challenges in efficient utilization and conversion of CO/syngas to electricity in an MFC is the mass transfer limitations of these sparingly soluble gases in the liquid phase. To counter this challenge, the following study evaluated the applicability of two different configurations of dense polymer silicone membrane systems for efficient CO transfer into the anodic liquid of an MFC. It was hypothesized that the flat silicone membrane system and the thin wall silicone tubing could efficiently enhance CO gas to liquid mass transfer with reduced reactor volumes, thereby allowing for increased CO transformation efficiency and improved volumetric power outputs.

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CHAPTER 3

Use of silicone membranes to enhance gas transfer during microbial fuel cell operation on carbon monoxide

3.1 Abstract

Electricity generation in a microbial fuel cell (MFC) using carbon monoxide (CO) or synthesis gas (syngas) as an electron donor has been demonstrated recently. A major challenge associated with CO or syngas utilization is the mass transfer limitation of these sparingly soluble gases in the aqueous phase. This study evaluated the applicability of a dense polymer silicone membrane and thin wall silicone tubing for improved CO transfer in MFCs. Replacing the sparger used in our previous study with the membrane systems for CO delivery resulted in improved CO transformation efficiency and MFC performance. A power output and CO transformation efficiency of up to 18 mW L_R^{-1} (normalized to anode compartment volume) and 98 %, respectively, was obtained. The use of membrane systems offers the advantage of improved mass transfer with reduced reactor volume, thus increasing the volumetric power output of MFCs operating on a gaseous substrate such as CO.

Key words: microbial fuel cell; carbon monoxide; silicone membrane; gas transfer

3.2 Introduction

Biomass represents a promising source of renewable energy (Song, 2002). The gasification of biomass at high temperatures leads to the production of synthesis gas (syngas), which mainly consists of carbon monoxide (CO) and hydrogen (H_2). Syngas can be converted to liquid (ethanol, methanol, butanol, etc.) or gaseous (H_2, methane) fuels by catalytic or microbial conversion (Henstra et al., 2007; Munasinghe & Khanal, 2010; Sipma et al., 2006). Syngas produced by partial oxidation using air, has a varying composition and a low heating value. It is often utilized for electricity generation by internal combustion (Bridgwater, 2006), with intrinsically low conversion efficiency. Direct conversion of syngas to electricity in a fuel cell offers a cleaner and a more efficient process for electricity generation. However, fuel cells are sensitive to the changes in the syngas composition (Kim & Chang, 2009; Song, 2002) and fuel impurities such as sulfur compounds, with > 1 ppm sulfur leading to irreversible poisoning of the anode.

As an alternative, syngas could be converted to electricity in a microbial fuel cell (MFC) (Kim & Chang, 2009; Mehta et al., 2010). This process relies on the ability of an anaerobic consortium of electricigenic and carboxydotrophic microorganisms to produce electricity from CO and H_2. Such an MFC-based process might offer several advantages such as a high transformation efficiency, a broad range of operating conditions, high bio-catalytic activity and considerable resistance to poisoning by fuel impurities (Kim & Chang, 2009; Mehta et al., 2010).

Kim and Chang (2009) demonstrated electricity generation from CO in a two-stage reactor system, in which CO was first microbiologically converted to fermentation

products and the products were then fed to the second reactor (MFC). Instead of a two-stage process, we demonstrated electricity generation in a single chamber MFC directly fed with CO (Mehta et al., 2010). The maximum volumetric power output and CO transformation efficiency of the system was 6.4 mW L_R^{-1} and 53 %, respectively, and although comparable with current literature, this was relatively low. Based on experimental observations, Mehta et al. (2010), suggested that the performance of a CO-fed MFC could be increased by adopting an efficient method for gas to liquid transfer, while optimizing the anodic chamber and avoiding CO related inhibition of electricigenic microorganisms.

For sparingly soluble gases such as CO, the primary resistance to transport is in the liquid side of the film at the gas-liquid interface (Bredwell et al., 1999; Riggs & Heindel, 2006). Membrane systems offer an efficient and relatively inexpensive method for gas-liquid mass transfer with reduced reactor volumes (Munasinghe & Khanal, 2010; Scott & Hughes, 1996). Thin synthetic polymer membranes have been studied considerably for their mass transfer properties (Robb, 1968). Hence, either a dense polymer or a micro-porous membrane could be employed in an MFC depending on the characteristics of the gas and operating conditions of the process. In a dense polymer membrane, the gas is absorbed into the polymer at high pressure and is carried into the liquid (lower pressure side) by diffusion across the membrane (Yasuda & Lamaze, 1972). In contrast, the gas in a micro-porous system is transported into the liquid through the pore system rather than through the polymer (Côté et al., 1989). Dense polymer membranes, such as silicone membranes, offer an advantage over micro-porous membranes as they can be operated under high pressure conditions, which increase the concentration gradient and hence, the mass transfer rate (Côté et al., 1989). Silicone membranes have been reported to be ideal

for membrane based bubble-less aeration without vigorous mixing where a conventional system is unable to meet the O_2 requirements of a higher rate system (Ahmed & Semmens, 1992; Côté et al., 1989). They also find wide applicability in modified atmosphere storage for fruits and vegetables because of efficient regulation of the gas levels in the storage chamber due to their selective gas permeation properties (Gariépy et al., 1994). Though silicone membranes have not yet been extensively studied for CO transfer from gas to liquid, they have been reported to have much higher diffusion rates for gases in comparison to other hydrocarbon polymers (Robb, 1968). Therefore, it was hypothesized that silicone membranes could offer an efficient membrane based mass transfer system for CO.

The current study compares the flat silicone membrane and silicone tubing for CO transfer to the anodic liquid and demonstrates MFC operation on CO and syngas at an improved volumetric power output.

3.3 Materials and Methods

3.3.1 Membrane systems for CO transfer

The flat silicone membrane (SSP LLC, NY, USA) used in this study consisted of a fine nylon fabric (52-54 g/m^2) calendered with a thin uniform layer of silicone rubber (~90 µm). The fabric enhanced the mechanical properties of the membrane, while the silicone rubber was responsible for its gas permeation properties. A silicone tubing (VWR International LLC, Radnor, PA, USA) with a wall thickness of 9 µm and an external diameter of 0.076 mm was used. The total surface area for the silicone membrane and the tubing used in the tests was kept constant at 50 cm^2.

3.3.2 Volumetric mass transfer coefficient (K_La) estimation

The experimental set-up for determination of K_La consisted of a 50 mL test chamber custom made (Wolltech, Montreal, Canada) from Makrolon (polycarbonate) plates filled with tap water (Figs. 3.1A and 3.2A). Silicone tubing wound around a support was placed directly inside the test chamber as shown in Fig. 3.1A. For the silicone membrane an extra plate serving as the CO compartment was attached to the test chamber with the membrane acting as a wall between the two compartments as shown is Fig. 3.2A. The inlet of the CO compartment or the silicone tubing was connected to a CO bag, while the outlet was connected to a manometer. Both, the CO compartment and the tubing were maintained at a constant pressure throughout the experiment.

K_La values for CO were determined using the method described by Munasinghe and Khanal (2010b), introducing a step change in dissolved CO concentration and then evaluating the concentration as a function of time. For this condition, the desired CO flow rate was set using a peristaltic pump connected to a timer prior to the start of the experiment. A gas tight syringe was used to collect water samples from a sampling port in the liquid recirculation line at regular intervals until the dissolved CO concentration reached a steady state. The dissolved CO concentration was determined as described by Lide and Frederikse (1995). The sampled liquid was transferred into a hermetically sealed vial and placed in a 90 °C water bath for 10 min in order to achieve equilibrium between the liquid and the gas phase. The gas in the headspace of the vial was then analyzed as described below.

The K_La value was estimated based upon the mass balance equation $dC/dt = K_La\ (C_{ct} - C_t)$ (Munasinghe & Khanal, 2010b), where C_t is the dissolved CO concentration at time t and C_{ct} is the CO concentration in equilibrium between the gas-liquid phase. Assuming that C_{Ct} is constant at all times, there is no chemical reaction, and initially (at $t = 0$) there is no gas present in the test chamber, the solution of the mass balance equation yields:

$$\ln\left(\frac{C_{ct}-C_o}{C_{ct}-c_t}\right) = (\mathbf{K_L a})\ t \quad \text{.....} \quad (3.1)$$

Where: C_o is the initial dissolved CO concentration.

Equation 3.1 corresponds to a linear relationship, in which the slope of the line is the volumetric mass transfer coefficient, K_La (Munasinghe & Khanal, 2010b; Riggs & Heindel, 2006).

3.3.3 Medium composition, inoculum and analytical methods

The composition of the stock solution of nutrients in g/L was: 1.87 NH_4Cl, 14.81 KCl, 6.40 K_2HPO_4, 4.07 KH_2PO_4 and 0.415 yeast extract. The trace metal stock solution was prepared according to Tartakovsky et al., (2008), and contained (in g/L): 2 $FeCl_24H_2O$, 0.05 H_3BO_3, 50 $ZnCl_2$, 0.03 $CuCl_2$, 0.5 $MnCl_24H2O$, 0.5 $(NH_4)6Mo7O_244H2O$, 0.5 $AlCl_3$, 0.5 $CoCl_26H_2O$, 0.5 $NiCl_2$, 0.5 EDTA, and concentrated HCl (1 mL). The chemicals used were all of analytical grade. The stock solutions were sterilized and maintained at 4 °C until used. The MFC influent solution was prepared by adding 35 mL of the nutrient solution and 1 mL of the trace metal solution to 1 L of de-ionized water. This solution had a conductivity of 12 mS cm^{-1}. The MFC's were inoculated with 10 mL of anaerobic sludge

obtained from a food processing plant (Lassonde Inc., Rougemont, QC, Canada). The sludge was stored at 4 °C, and the pH of the sludge was 6.8-7.0.

Acetate and other volatile fatty acids (VFAs) were analyzed in an Agilent 6890 gas chromatograph (Wilmington, DE, USA) equipped with a flame ionization detector. The method details are provided in Tartakovsky et al. (2008). The pH and conductivity of the effluent were measured periodically. The volume of inflow and outflow gas was measured using bubble counters connected to glass U-tubes and interfaced with a data acquisition system.

The gas composition (H_2, CO, CH_4, O_2, N_2, and CO_2) was measured using an Agilent 6890 gas chromatograph (Wilmington, DE, USA) coupled to a thermal conductivity detector. Three hundred microliter of headspace sample was injected into a 4.5 m x 2 mm I.D. carboxen 1000 packed column from Sigma-Aldrich (St. Louis, MO, USA). The column was heated at 60 °C for 7 min, then raised to 225 °C at a rate of 60 °C per minute and maintained for 6 min. Argon was used as carrier gas. The injector and detector were maintained at 125 °C and 150 °C respectively.

3.3.4 MFC design, Operation and Characterization

MFC tests were conducted in an experimental set-up similar to that employed for K_La measurements. The 50 mL anodic compartment contained the 5 mm thick graphite felt anode measuring 10 cm x 5 cm (Speer Canada, Kitchener, ON, Canada). A cathode was attached to one of the sides of the anodic compartment (Figs. 3.1B and 3.2B). All MFC tests were carried out using a carbon paper cathode containing 0.5 mg cm^{-2} Pt as the catalyst, (GDE LT 120EW, E-TEK Division, PEMEAS Fuel Cell Technologies, Somerset,

NJ, USA), which was separated from the anode by a piece of J-cloth, such that the estimated distance between the electrodes was 0.7-1 mm. The cathode was prepared as described in Mehta et al. (2010). The MFCs were equipped with lines for influent, effluent, liquid recirculation, gas exit and entry as shown in figures 3.1B and 3.2B. Medium was prepared and fed to the MFC at a rate of 75 mL/day using a peristaltic pump. The MFC's were operated at 33–35 °C by heating the re-circulating liquid. The temperature was monitored and controlled by means of a thermocouple placed in the anodic chamber and a temperature controller (Model JCR-33A, Shinko Technos Co., Ltd., Osaka, Japan).

For the silicone membrane MFC, a 10 L gas bag (Control Concepts Inc., Calgary, Alberta, Canada) was used for continuous supply of CO. In the silicone tubing tests, the line for gas entry was connected to a CO cylinder and the CO flow rate was controlled by a flow meter (Alicat Scientific, USA). Several CO flow rates were tested in order to estimate and maximize CO consumption efficiency. The gas flow rates are expressed in L of gas fed per L of anodic chamber volume per day (L L_R^{-1} d^{-1}). CO flow rates of 4 and 6 L L_R^{-1} d^{-1} were tested for silicon membrane MFC and CO flow rates of 2, 3 and 4 L L_R^{-1} d^{-1} were tested for silicon tubing MFC. Each flow rate was tested for at least 7 days. In addition to CO tests, syngas operation was simulated by attaching a 10 L H_2 bag to the CO line. A flow rate of 4 L L_R^{-1} d^{-1} was used for CO and H_2 into the silicon membrane MFC and 3 L L_R^{-1} d^{-1} was used for silicon tubing MFC. The gas flow rates tested in this study were similar to that tested in the previous study (Mehta et al., 2010). This was done to allow for a direct comparison of the volumetric power output and CO transformation efficiency achieved for the MFCs in this study to that achieved for the sparger installed MFC (Mehta et al., 2010).

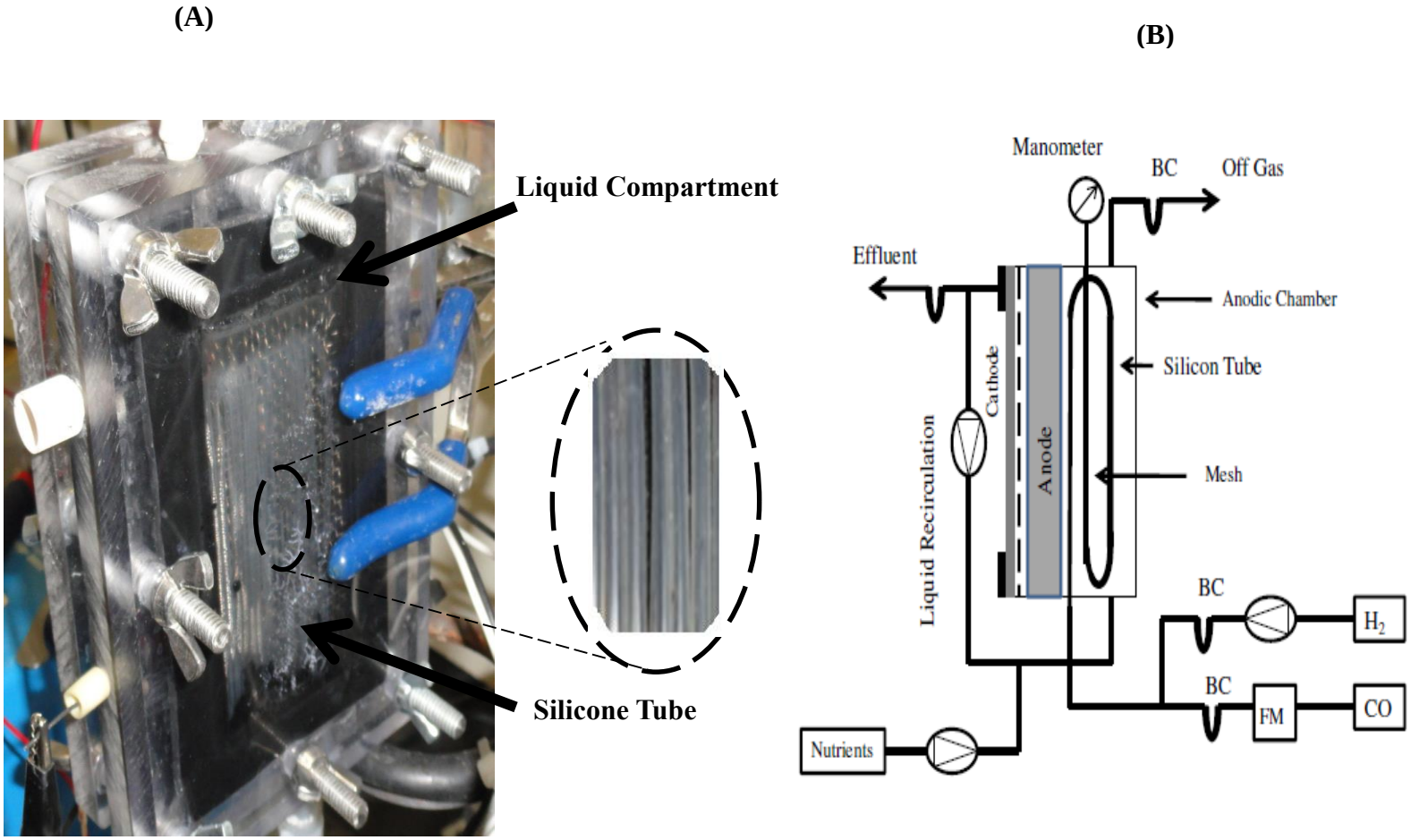


Figure 3.1 (A) Experimental set-up for the determination of volumetric mass transfer co-efficient ($\mathbf{K_L a}$) for silicone tubing and (B) Diagram of the experimental setup of silicone tubing incorporated MFC, where BC represents the bubble counters.

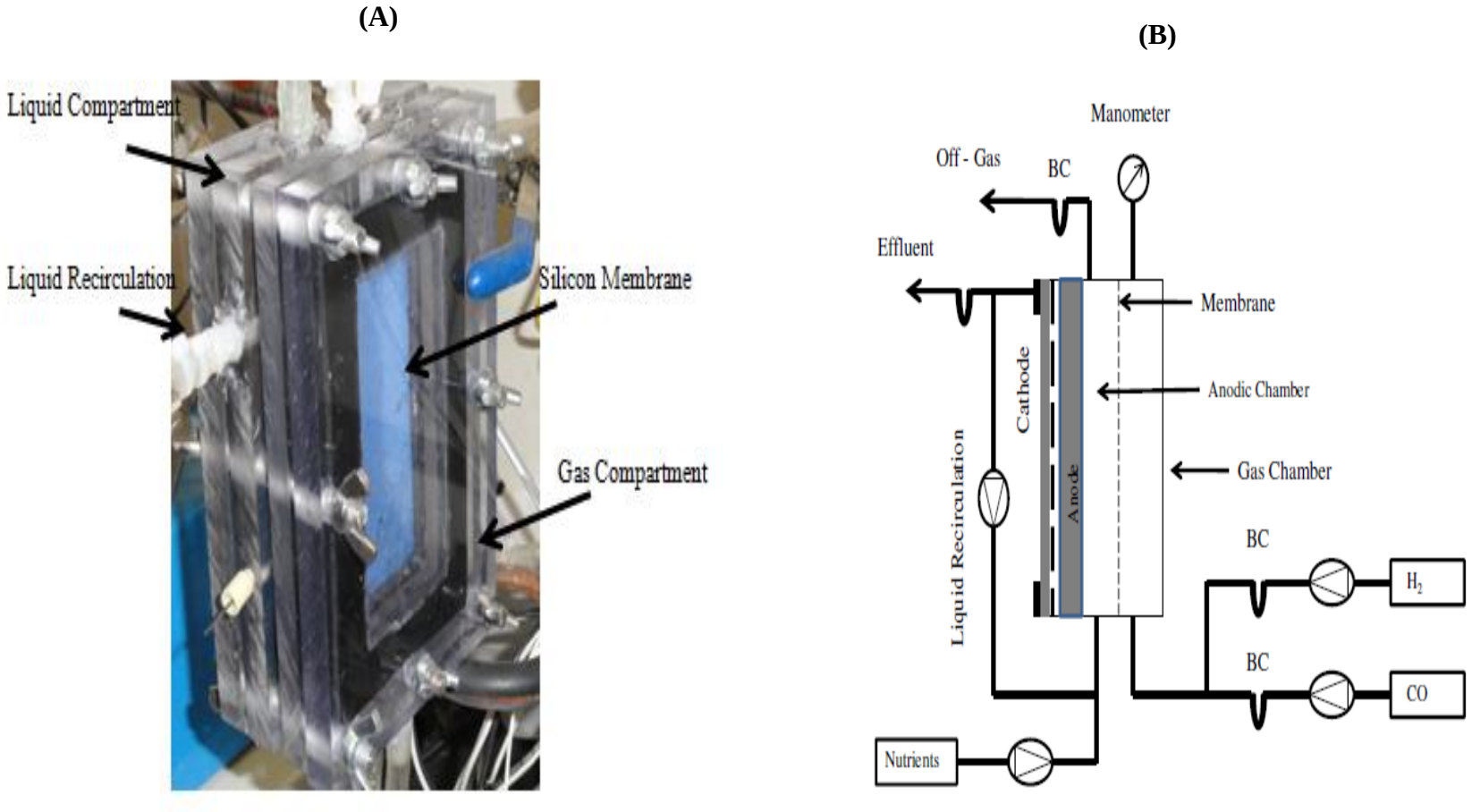


Figure 3.2 (A) Experimental set-up for the determination of volumetric mass transfer coefficient ($\mathbf{K_L a}$) for silicone membrane and (B) Diagram of the experimental setup of silicone membrane incorporated MFC, where BC represents the bubble counters.

MFC voltage was measured on-line at 20 min intervals using a data acquisition system (Labjack U12, Labjack Corp., Lakewood, CO, USA). Polarization tests were carried out at the end of each test using the following method. First, the open circuit voltage (OCV) was measured by disconnecting external resistance for 30 min. The external resistance was re-connected and progressively decreased in 8-11 steps with an interval of 10 minutes between each step to allow for voltage stabilization. The 10 min intervals provided a steady state voltage, while avoiding significant changes in the carbon source concentration in the anodic liquid. Output voltage measurements were conducted at the end of each interval. The measurements were started at 5000 Ω and terminated at 15 Ω. These measurements were used to generate polarization (voltage vs. current density) and power (output power vs. current density) graphs (Logan, 2008; Mehta et al., 2010). The linear part of the polarization curve produced was used to estimate the total internal resistance (Logan, 2008). The open circuit potentials for anode and cathode were measured using a standard Ag/Agcl electrode (0.222 V vs NHE).

The Coulombic efficiency (CE) of the MFC was estimated as (Mehta et al., 2010):

$$CE = \frac{I \cdot \Delta t \cdot M_{CO}}{F \cdot n \cdot (M_{CO,in} - M_{CO,out})} 100\ \% \quad \text{..... (3.2)}$$

Where, I is the average measured current (A), Δt is the time interval during which current was measured (s), M_{CO} is the molar mass of CO (g/mol), F is the Faraday constant (C/mol-e^-), n is the number of electrons transferred (mol-e^-/mol), $M_{CO,in}$ is the amount of CO supplied (g) to the MFC during Δt, and $M_{CO,out}$ is the amount of CO (g) recovered in the off-gas line during Δt.

The absence of power generation from the nutrients present in the influent stream (i.e. yeast extract) has been demonstrated earlier (Mehta et al., 2010). A low voltage output of 3- 4 mV (R_{ext} = 500 Ω, P_{out} < 0.02 x 10^{-3} mW) was observed when the MFC was fed only with the solution of nutrients and microelements in the absence of CO-feeding for 1 week (Mehta et al., 2010). In this study, at start-up the MFCs were operated under a constant external resistance and comparatively low CO flow rate. Once a steady power output was observed, the CO flow rate was stepped up and the external resistance was optimized using the Perturbation and Observation MPPT algorithm (Woodward et al., 2009). The external optimized resistance using the online algorithm was always found to be within the range of ± 20 Ω to that of the internal resistance obtained from the polarization curves. The effluent and anodic off-gas compositions were analyzed periodically. In order to estimate the dissolved CO concentration levels corresponding to optimum performance, the dissolved CO concentration in the anodic liquid was measured regularly. At each tested flow rate, polarization tests were performed to estimate the MFC internal resistance and maximum power output (P_{max}^{pt}). Also, the average (P_{av}) and maximum operational power output (P_{max}^{op}) observed during the course of MFC operation at a particular CO flow rate is also reported. Notably, P_{av} takes into account the fluctuations in power output.

3.4 Results and Discussion

3.4.1 Estimation of K_La

The K_La values for CO were calculated using the analytical solution of the dissolved CO material balance (Eq. 3.1). The silicone membrane and tubing were operated in a dead-end mode. This mode of operation allowed for an elevated gas pressure in the system due

to gas build up, which increased the concentration gradient and hence the mass transfer of CO (Ahmed & Semmens, 1992). The CO K_La for silicone membrane and tubing were estimated to be 0.63 ± 0.1 h^{-1} and 0.76 ± 0.2 h^{-1}, respectively. For an optimum performance of a CO-fed MFC, the rate of CO transfer needs to be matched to that of its consumption, since an excessive transfer would lead to CO-related inhibition of carboxydotrophic and electricigenic microorganisms (Henstra et al., 2007; Mehta et al., 2010; Sipma et al., 2006). The CO transfer rates obtained for the silicone membrane and silicone tubing sufficiently matched to the gas transfer requirements for the CO-fed MFC in the study of Mehta et al. (2010); confirming the feasibility of using silicone membrane and tubing for CO transfer in MFCs. The K_La value for silicone tubing was comparably higher than for the membrane, conceivably due to the difference in their thickness (Ahmed & Semmens, 1992). Comparable CO K_La in the range of 0.4 to 1.1 h^{-1} for composite hollow fiber membrane modules has been recently reported (Munasinghe & Khanal, 2010b). To our knowledge, no other literature evaluating the mass transfer of CO (gas to liquid) through membrane systems is currently available.

3.4.2 MFC tests

The operation of the silicone membrane MFC was started with a CO flow rate of 2 L L_R^{-1} d^{-1} and an external resistance of 1089 Ω. After a delay of 2 weeks, a steady voltage of 203 mV corresponding to a power output of 0.76 mW L_R^{-1} was observed. At this point, the CO flow rate was stepped up to 6 L L_R^{-1} d^{-1} and the external resistance was now optimized using the online optimization algorithm. After 9 days of operating the MFC at this flow rate, an average (P_{av}) and maximum operational power output (P_{max}^{op}) of 6 mW

L_R^{-1} (Fig. 3.3) and 11.2 mW L_R^{-1} (Table 3.1), respectively were observed. A maximum power output (P_{max}^{pt}) of 10.1 mW L_R^{-1} with an internal resistance of 175 Ω (Table 3.1) was estimated based on the polarization curves (Fig. 3.4). An open circuit voltage (OCV) value of 576 mV was observed with the cathode and anode OCP values lying in the range of 200 and 210 mV and between 350 and 385 mV, respectively. Flow rates higher than 6 L L_R^{-1} d^{-1} were not tested as it was found to impede the MFC output in our earlier study (Mehta et al., 2010). As mentioned earlier, for the optimum performance of a CO-fed MFC, the rate of gas transfer must match the rate of its consumption. The relatively high levels of dissolved CO concentration at higher CO flow rates leads to the inhibition of carboxydotrophic and electricigenic microorganisms, thereby impeding the performance of the MFC (Henstra et al., 2007; Mehta et al., 2010). Decreasing the CO flow rate to 4 L L_R^{-1} d^{-1} increased P_{av} and P_{max}^{op} to 8.4 mW L_R^{-1} (Fig. 3.3) and 19.3 mW L_R^{-1} (Table 3.1), respectively. Also, the CO transformation efficiency of the system increased to 74.2 % (Table 3.1). The P_{max}^{pt} increased to 18 mW L_R^{-1} (Fig. 3.4) and the internal resistance decreased to 80 Ω (Table 3.1). This increase in performance was plausibly due to a drop in dissolved CO concentration levels as given in Table 3.1.

As the maximum power output for silicone membrane MFC was observed at a CO flow rate of 4 L L_R^{-1} d^{-1}, the syngas test was performed by feeding both CO and H_2 at flow rate of 4 L L_R^{-1} d^{-1}. After an initial drop of power output to 2 mW L_R^{-1}, the power output progressively increased to 10 mW L_R^{-1} within 4 days of starting of the syngas test. The anodic chamber had to be opened at this point due to technical problems, which resulted in

exposure of the anode to oxygen. The MFC was restarted with syngas feeding and a recovery period of 1 week was required before any activity could be observed. The power output later increased to 8 mW L_R^{-1} . The performance of the MFC was plausibly hampered by the inhibition of the anaerobic carboxydotrophic and electricigenic microorganisms upon exposure to O_2, which was ascertained by a decrease in anode OCP to between 220-240 mV. Carboxydotrophs are strict anaerobes that are sensitive to O_2 (Oelgeschlager & Rother, 2008). A similar decrease in the output of a CO-fed MFC upon exposure of the anode to oxygen was also observed in our previous study (Neburchilov et al., 2011). Continued operation of the MFC on syngas for 15 days produced P_{max}^{pt} and P_{av} of 7 mW L_R^{-1} (Table 3.1) and 7.2 mW L_R^{-1} (Fig. 3.3) respectively, which were approximately 65 % higher than that previously reported for a syngas fed MFC (Mehta et al., 2010).

Notably, the fluctuations in the power output observed during the operation of silicone membrane MFC was likely caused by trace amounts of O_2 in the gas bags used to feed the MFC with CO and H_2. An increase in power output was always observed within 24 h of replenishing the gas bag. In order to obtain the best estimation of MFC performance the polarization tests were always performed within 24-30 h of replenishing the gas bags when the MFC performance was observed to be most stable.

Table 3.1 Performance of silicone membrane incorporated MFC at different CO flow rates.

Test	L $(L_R)^{-1}d^{-1}$	Power output (mW/L_R) P^{op}_{max}	Power output (mW/L_R) P^{pt}_{max}	R_{int} (Ω)	CO transformation (%)	Effluent acetate (mg/L)	H_2 (%)	CH_4 (%)	CO (%)	CE (%)	CO conc. (mg/L)
1	6	11.2	10.1	175	67.8±2	46.2	4.8	15.3	62.5	7.0	9.2
2	4	19.3	18	85	74.2±3.1	18.4	3.1	11.1	55.1	10.7	7.2
3*	4*	8	7	170	57.2±4.4	34.7	22	11.5	44.5	6.8	7.1

*Syngas test (CO and H_2 fed simultaneously at the indicated flow rate)

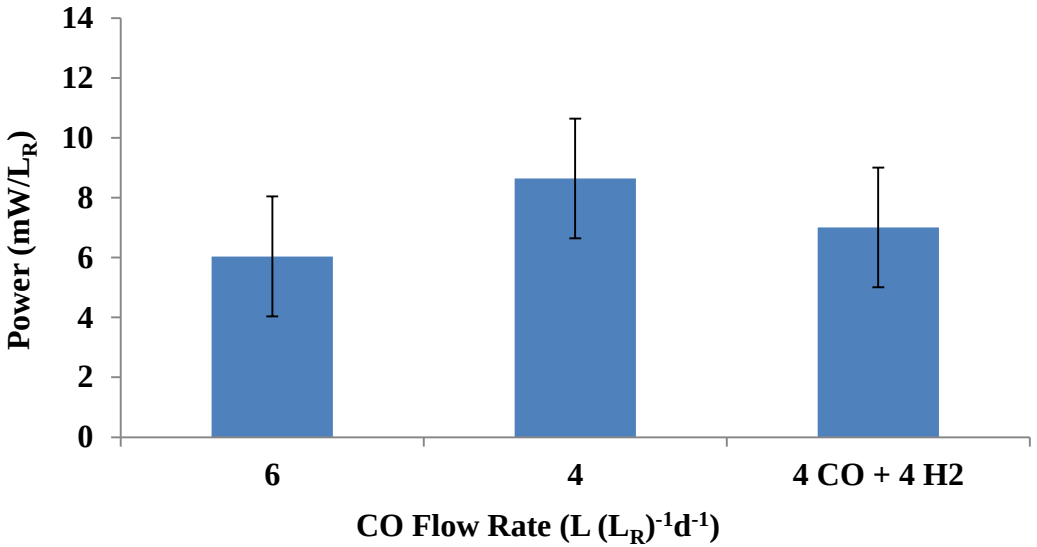


Figure 3.3 Average volumetric power output for silicone membrane incorporated MFC at different CO flow rates. The error bars represent the calculated standard deviation for the data points.

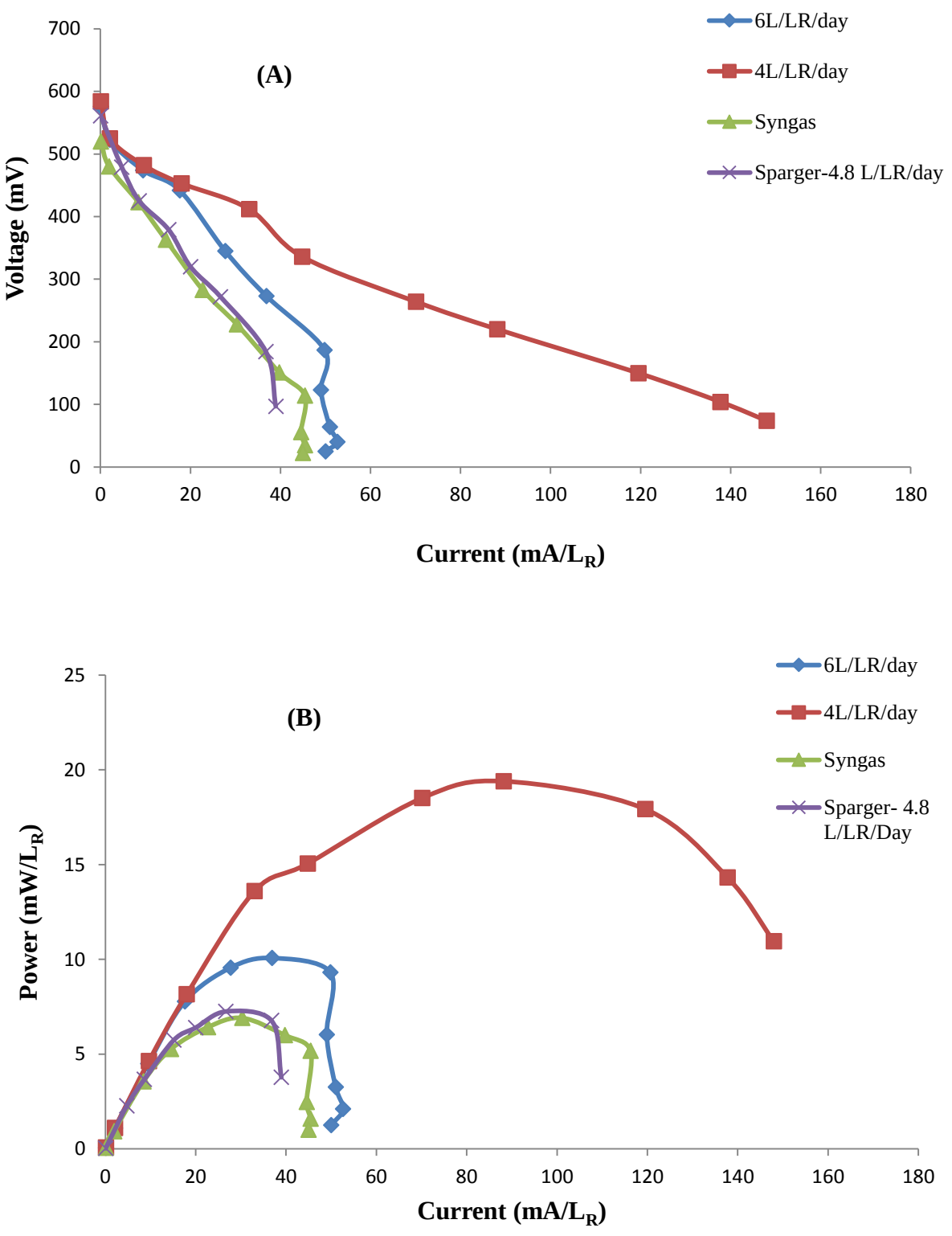


Figure 3.4 Polarization (A) and power (B) curves obtained for flat silicone membrane incorporated MFC, where sparger represents the values obtained for the sparger installed MFC maximized at the flow rate of 4.8 L L_R^{-1} d^{-1} (Mehta et al., 2010).

In general, the performance of a silicone membrane MFC was comparatively better than that observed for the sparger equipped MFC in the previous study (Mehta et al., 2010). The maximum volumetric power outputs, P_{av} and P_{max}^{pt} at the CO flow rate of 4 L L_R^{-1} d^{-1} were approximately 35 % and 180% higher than that previously reported (Kim & Chang, 2009; Mehta et al., 2010). Also, the CO transformation efficiency increased by 20 %. The CE obtained during CO feeding was comparable to that reported for the sparger equipped MFC (Mehta et al., 2010).

For studying the performance of the silicone tubing MFC, a silicone tube wound around a support (stainless steel mesh) was placed next to the anode. Also, in order to avoid the problem of fluctuation in power output due to O_2 diffusion in the gas bags, the CO line was connected directly to a CO cylinder. The tests were performed with the same anode as that used in the silicone membrane tests, thus the variations in power output resulting from differences in the microbial populations were avoided.

The CO transformation efficiency in the silicon tubing MFC was comparatively higher than that observed for the silicone membrane MFC, with the maximum power outputs, P_{max}^{op} and P_{max}^{pt} observed at a CO flow rate of 3 L L_R^{-1} d^{-1} (Table 3.2). TheP_{av} and P_{max}^{pt} and were 10 mW L_R^{-1} (Fig. 3.5) and 13 mW L_R^{-1} (Fig. 3.6) respectively, which were approximately 103 and 60 % higher than that observed in previous study (Mehta et al., 2010). Also, a CO transformation efficiency of 92 % was achieved (Table 3.2) Though the maximum CO transformation efficiency of 98.5 % (Table 3.2) was observed at a flow rate of 2 L L_R^{-1} d^{-1}, the power output (Fig. 3.6) was comparatively lower as the process was plausibly limited by low acetate concentrations. The electricity generation in a CO-fed

MFC predominantly takes place by the conversion of CO to acetate by the acetogenic carboxydotrophic microorganisms followed by the oxidation of acetate by CO tolerant electricigenic microorganisms. Low acetate levels at low CO flow rates limit electricity generation in a CO-fed MFC (Mehta et al., 2010).

Silicon tubing MFC operation on syngas was tested by feeding CO and H_2 at flow rate of 3 L L_R^{-1} d^{-1}. A P_{max}^{pt} of 8.1 mW L_R^{-1} (Fig. 3.6) and a P_{av} of 6.7 mW L_R^{-1} (Fig. 3.5) were observed, which were approximately 92% and 60% higher than that previously reported (Mehta et al., 2010). Fluctuations in power output of silicon tubing MFC were less intense than those observed for silicone membrane MFC. However, the O_2 levels in the anodic chamber of silicone tubing MFC increased with the installation of the H_2 bag, impeding the carboxydotrophic activity, which can be inferred from the low CO transformation efficiency (41.5 %) and a decrease in anode OCP (250-270 mV) observed during the syngas test.

Overall, the volumetric power output of silicone tubing MFC was comparable to that obtained for silicone membrane MFC, but the CO transformation efficiency for silicone tubing MFC was considerably higher due to better CO gas to liquid transfer capability of silicone tubing as reflected by the higher CO K_La value for the tubing than for the silicone membrane. Consequently, the optimum level of dissolved CO was achieved at a lower CO flow rate. The power output was maximized at dissolved CO levels of 7.8 to 8 mg/L (Table 3.2).

Table 3.2 Performance of silicone tubing incorporated MFC at different CO flow rates.

Test	L $(L_R)^{-1}d^{-1}$	Power output mW/L_R P^{op}_{max}	Power output mW/L_R P^{pt}_{max}	R_{int} (Ω)	CO transformation (%)	Effluent acetate (mg/L)	H2 (%)	CH_4 (%)	CO (%)	CE (%)	CO conc. (mg/L)
1	4	12.2	11.7	150	88±2.3	74	4.7	9.4	39.3	9.3	9.1
2	3	14	13	75	92±1	58	2.1	9.3	33.2	16.3	7.8
3	2	4	3.1	200	98.4±1	6.1	5.3	15	12.5	7.0	3.1
4*	3*	6.3	8.1	130	41.4±2.6	51.4	26	3.3	48.4	8.8	8.4

*Syngas test (CO and H_2 fed simultaneously at the indicated flow rate)

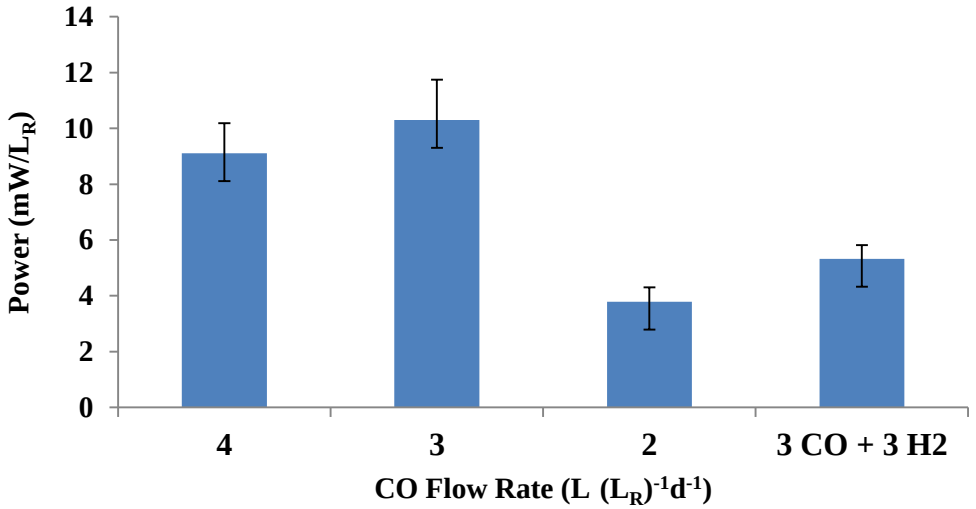


Figure 3.5 Average volumetric power output for silicone tubing incorporated MFCs at different CO flow rates. The error bars represent the calculated standard deviation for the data points.

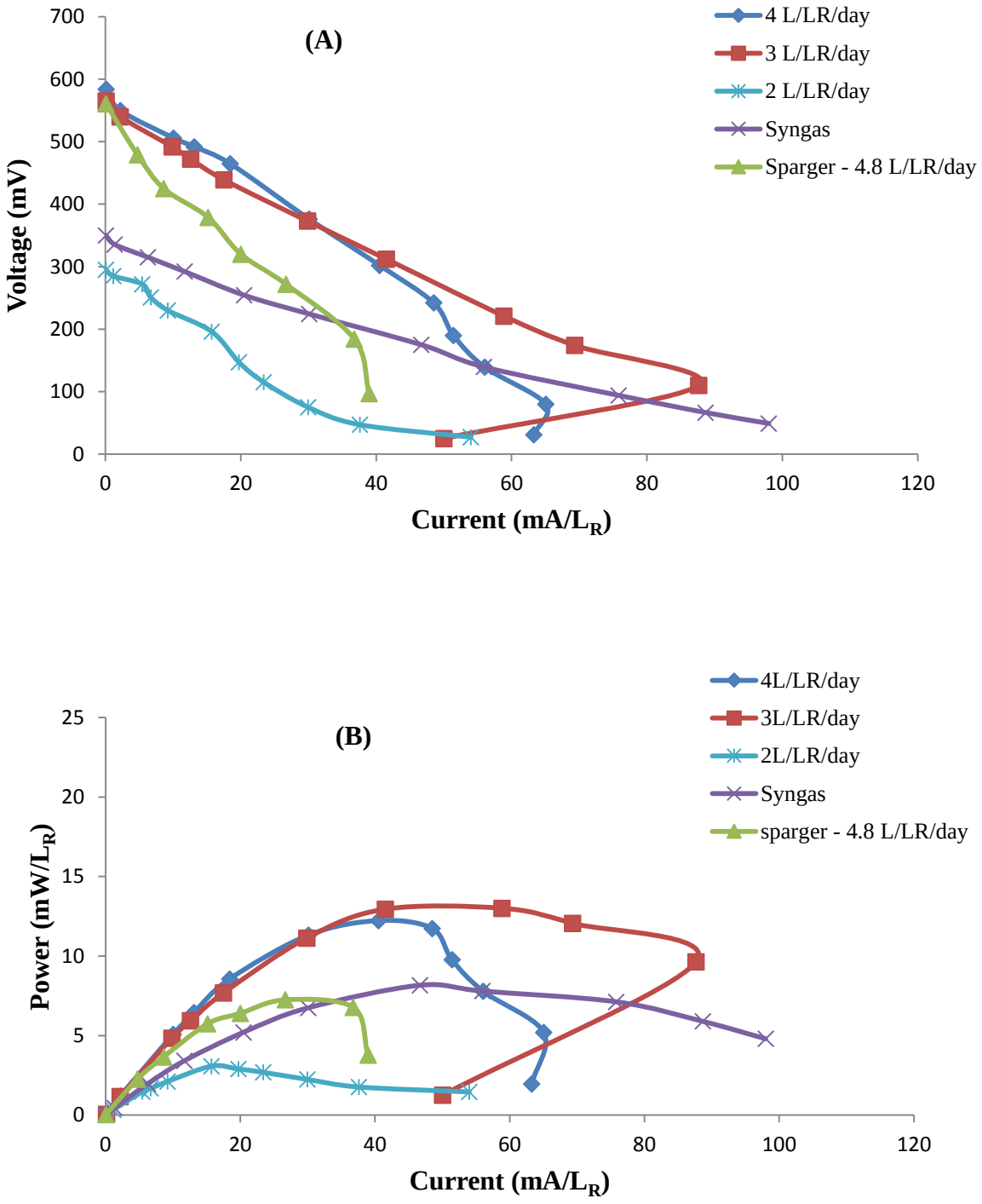


Figure 3.6 Polarization (A) and power curves (B) obtained for silicone tubing incorporated MFC, where sparger represents the values obtained for sparger installed MFC maximized at the flow rate of 4.8 L L_R^{-1} d^{-1} (Mehta et al., 2010)

The maximum current density achieved for silicone membrane and silicone tubing MFCs were in a range of 1.0-1.4 A m^{-2} and 0.7-0.9 A m^{-2}, respectively. Acetate-fed MFCs have been reported to have a similar current density of 1.1-2.0 A m^{-2} and a power output of 10-100mW L_R^{-1} (Logan et al., 2006; Lovley, 2008; Martin et al., 2010; Neburchilov et al., 2011; Pinto et al., 2010). Such current densities could be considered low for a hydrogen fuel cell, however they are sufficient to consider an MFC as a feasible technology for electricity production from organic waste (Logan, 2010). In our study, the anode occupied 25 mL of the anodic chamber. By recalculating the volumetric power output per anode volume, a power output of 36 mW/L_{anode} for silicone membrane (at 4 L L_R^{-1} d^{-1}) and 26 mW/L_{anode} for silicone tubing (at 3L L_R^{-1} d^{-1}) was obtained, which implies that further design improvements/optimization of cathode and the anodic chamber can result in a higher power ouput for a CO-fed MFC.

The results of VFA and off gas analysis (Fig 3.7) for silicone membrane and silicone tubing tests were in accordance with previous observations (Kim & Chang, 2009; Mehta et al., 2010). The effluent measurements demonstrated the predominance of acetate as the major metabolite with propionate, methanol and ethanol being present only in trace amounts. The concentration of acetate increased as CO flow rate increased (Table 3.1 and 3.2). Degradation products such as H_2 and CH_4 were detected in the anode off-gas. Based on the analysis of metabolic products, Mehta et al.,(2010) hypothesized that the production of electricity in CO-fed MFC proceeds through multi-step concurrent pathways. The conversion of CO to acetate followed by the oxidation of acetate by CO tolerant electricigenic microorganisms was hypothesized to be the most prominent pathway. The

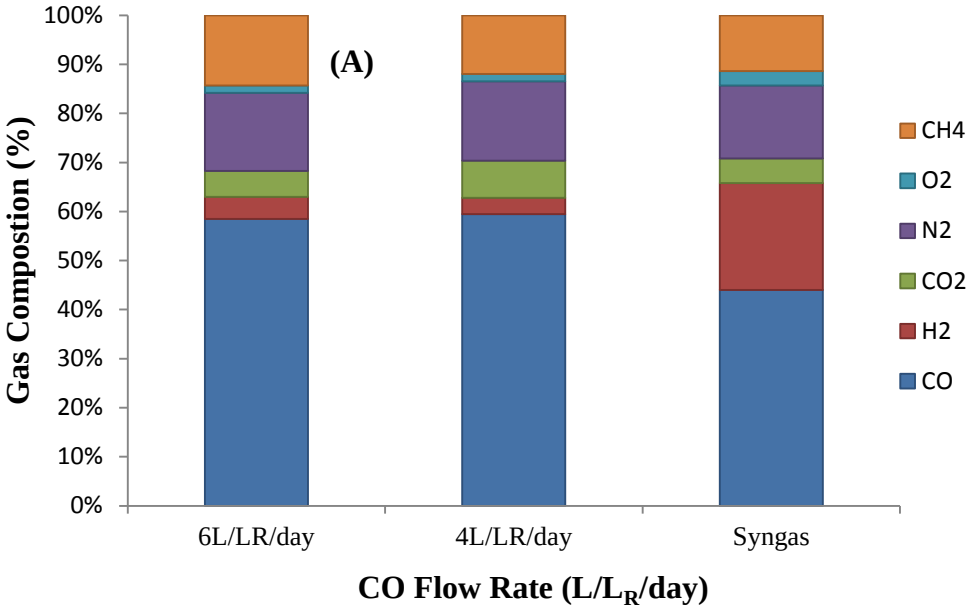
following stoichiometric equations describe this pathway (Hussain et al., 2011; Mehta et al., 2010):

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \quad (3.3)$$

$$CH_3COOH + 2H_2O \rightarrow 2CO2 + 8e^- + 8\ H^+ \quad (3.4)$$

The presence of H_2 in the off-gas when the MFC was operated on CO was indicative of hydrogenogenic activity. It was hypothesized that H_2 was used as an electron donor for electricity generation by the electricigenic microorganisms. Electricity generation in an MFC fed with H_2 has previously been demonstrated (Bond & Lovley, 2003). This particular pathway can be expected to have profound relevance for MFC operation on syngas (Mehta et al., 2010).

The CE of our system was invariably low, conceivably due to the concomitant production of CH_4 and other degradation products (Mehta et al., 2010; Sipma et al., 2003). In the study of Sipma et al. (2003), CO conversion in some cases led to the production of acetate and CO_2 and to CH_4 and CO_2 in others. More importantly it was noticed that acetate seemed to serve as the main intermediate for CH_4 production, implying that much higher CE for a CO-fed MFC can be achieved if the formation of CH_4, H_2 and other degradation products can be kept to a minimum. Mehta et al., (2010) reported a CE of 33 % for a CO-fed MFC when the calculations were modified to take into account the consumption of fed CO for the production of CH_4, H_2 and acetate that was not used for electricity production. This CE is only marginally lower that reported for acetate MFCs with comparable designs (Logan, 2008).



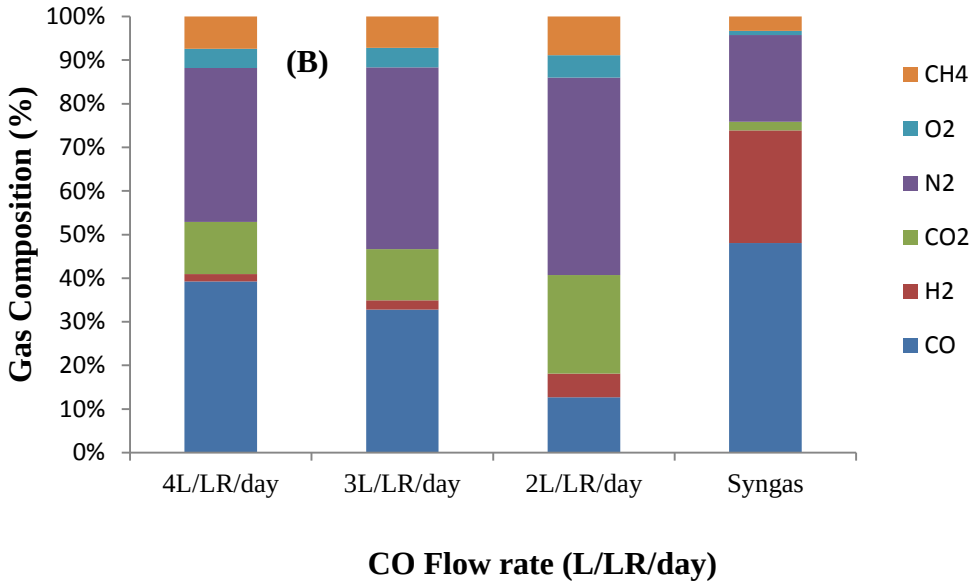


Figure 3.7 Anodic off–gas composition for (A) silicone membrane and (B) silicon tubing incorporated MFC.

Overall, the operation of a silicone membrane and silicone tubing MFCs clearly demonstrated that the performance of a CO-fed MFC can be substantially increased by adopting efficient mass transfer techniques. In our earlier study (Mehta et al., 2010), a sparger was used for gas to liquid transfer of CO. The installation of the sparger required not only the doubling of the anode compartment volume (100 mL) but also much higher CO flow rates to achieve the desired dissolved CO levels due to mass transfer limitations; resulting in considerable gas losses and a low CO transformation efficiency. In this study, with the installation of a silicone membrane or tubing, an efficient gas to liquid transfer was achieved with a reduced anodic volume (50 mL), which considerably increased the volumetric power output along with a significant increase in CO transformation efficiency.

Interestingly, we observed biofilm formation on the surface of silicone membrane (facing the liquid) and also on the tubing. Biofilm formation on membrane surfaces has been widely reported. In the patent held by Hickey et al., (2008) a membrane bioreactor was employed for the conversion of CO or syngas to ethanol and other liquid products. The membrane concurrently served as support for biofilm growth. Biofilm formation on the surface of silicone membranes in membrane bioreactors for waste gas and water treatment has been reported (Kumar et al., 2008; Reij et al., 1998). This phenomenon of biofilm formation on silicone membrane surfaces is quite pertinent to CO-fed MFCs, where a biofilm formation on membrane surface may increase the density of acetogenic carboxydotrophic microorganisms in the anodic chamber and thereby increasing the acetate concentration and CO transformation efficiency.

CO and syngas-fed MFCs offer a promising technology that has a number of advantages over polymer electrolyte membrane fuel cells (PEMFC) or solid oxide fuel cells (SOFC), as MFCs do not require noble catalysts at the anode nor are they very sensitive to fuel impurities as it has been previously reported in the case of PEMFC and SOFC (Kim & Chang, 2009; Song, 2002). The MFC based process can be used to combine electricity production with hydrogen extraction from syngas or in combination with syngas biotransformation to valuable energy carriers, such as butanol (Pengmei et al., 2007).

3.5 Conclusion

This study demonstrated the feasibility of using dense polymer membranes such as silicone membranes and thin wall silicone tubing for efficient mass transfer of CO into a MFC. Thin silicone membranes and tubing can be easily incorporated into an MFC without requiring extensive changes in its design. A P_{max}^{pt} of 13 mW L_R^{-1} and 18 mW L_R^{-1} was achieved in silicone tubing and membrane installed MFC respectively, which was approximately 103 % and 180 % higher than that previously reported. Also, much higher CO transformation efficiencies in the range of 75 to 99 % were obtained. Future studies might focus on testing other gas diffusion membranes with a high affinity for CO. Improved gas transfer demonstrated by the membrane-based systems, allows for focusing on the process microbiology, which could lead to the isolation of novel anodophilic carboxidotrophic microorganisms.

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Connecting text

The improved CO transformation efficiency and volumetric power output of mesophilic CO-fed MFCs in Chapter 3 clearly established the applicability of silicone membrane systems for efficient CO transfer into the anodic liquid of MFCs with reduced reactor volumes.

With an efficient gas delivery system now in place, the following study focused on elucidating the microbiology and biotransformation pathways driving electricity production from CO/syngas in an MFC. Culture independent finger printing technique such as denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified 16S rDNA fragments was utilized for identification of anodic microbial communities of two mesophilic CO-fed MFCs. The identified microbial communities along with the analysis of the metabolic products were used for mapping the biotranformation pathways driving electricity production from CO/syngas.

CHAPTER 4

Population analysis of mesophilic microbial fuel cells fed with carbon monoxide

4.1 Abstract

Electricity generation in a microbial fuel cell (MFC) fed with carbon monoxide (CO) and synthesis gas (syngas) has been recently demonstrated. However, the microbial ecology of this system has not yet been described. In this work the diversity of the microbial community present at the anode of CO-fed MFCs was studied by performing Denaturing Gradient Gel Electrophoresis (DGGE) analyses. The identified microorganisms belonged to the genera *Geobacter*, *Desolfovibrio*, *Clostridium*, *Methanobacterium*, *Methanofollis* and *Methanosaeta*. The presence of *G. sulfurreducens* suggested its tolerance to CO, which was confirmed by growing *G. sulfurreducens* with acetate under a 100% CO atmosphere. This observation, along with the identification of acetogens, supports the hypothesis of the two-step process in which CO is converted to acetate by the carboxidotrophic bacteria and acetate is then oxidized by CO-tolerant electricigenic bacteria to produce electricity.

Keywords: MFC; Carbon monoxide; *Geobacter sulfurreducens*.

4.2 Introduction

Studies on CO and syngas-fed MFCs have so far concentrated on the optimization of the system design (Hussain et al., 2011a; Neburchilov et al., 2011), which have increased the CO transformation efficiency and volumetric power output to 98% and 18 mW L_R^{-1}, respectively. Given that the electricity generation in an MFC is governed by the biological activity (Lovley, 2006), understanding of the metabolic pathways and microorganisms involved in driving electricity generation in a CO-fed MFC is critical for achieving high power densities. Electricity generation from CO till now has been demonstrated in an MFC seeded with a mixed microbial population of an anaerobic sludge (Hussain et al., 2011a). Based on the analysis of the metabolic products, Mehta et al.(2010) hypothesized that the production of electricity in a CO-fed MFC proceeds through multi-step concurrent pathways. The conversion of CO to acetate, followed by the oxidation of acetate by CO-tolerant electricigenic microorganisms was hypothesized to be the most prominent pathway. The presence of H_2 in the anodic off-gas when the MFC was fed solely with CO was indicative of hydrogenogenic activity. It was hypothesized that H_2 also served as an electron donor for electricity generation by electricigenic microorganisms. Finally, the possibility of direct electron transfer to the anode by metal-reducing carboxydotrophic microorganisms was also discussed. The primary objective of the study presented below was to characterize the microbial populations prevalent in the anodic chamber of the two previously described mesophilic CO-fed MFCs (Hussain et al., 2011a; Mehta et al., 2010) by denaturing gradient gel electrophoresis (DGGE) of PCR amplified 16S rDNA fragments. An acknowledged shortcoming of this molecular technique is that some of the essential species may not be

detected on the DGGE patterns (Ercolini, 2004). Nevertheless, the DGGE of PCR amplified 16S rDNA fragments remains a widely used molecular technique for rapid assessment of the microbial communities present in complex samples, such as MFC anodes (Logan, 2008).

4.3 Materials and Methods

4.3.1 MFC design and operation

The anode samples (graphite felt) were taken from the CO-fed MFC used in our previous study of Mehta et al.(2010) (hereby, referred to as MFC1) and the CO-fed MFC described in Chapter 3 (Hussain et al.,2011a; hereby referred to as MFC2). Both MFCs were similar in design and operation, with the only difference being in the method of CO delivery. In MFC1, a sparger was used for CO delivery in the anodic compartment, whereas in MFC2, a silicone membrane system was employed for CO transfer. With the installation of silicone membrane, an enhanced CO gas to liquid mass transfer was achieved with reduced anodic volume, which considerably increased the volumetric power output along with a significant increase in CO conversion efficiency. The MFCs were inoculated with 10 mL of anaerobic mesophilic sludge (Lassonde Inc, Rougemont, QC, Canada) and operated for a period of over 3 months at mesophilic temperatures (30-35 °C). Prior to use, the inoculum sludge was stored at 4 °C and the pH of the sludge was between 6.8 to 7.0. A solution of trace metals was prepared and fed to the MFCs at a rate of 75 mL d^{-1}. The trace metal stock solution was prepared as described by Tartakovsky et al. (2008).

4.3.2 Analytical methods

Volatile fatty acids (VFAs) and anodic off-gas composition were measured as described in Tarkakovsky et al. (2008) and Guiot et al. (2011). Briefly, VFAs such as acetate, propionate and butyrate, were analyzed using an Agilent 6890 gas chromatograph (Agilent Technologies Inc, Santa Clara, CA, USA) equipped with a flame ionization detector. The off-gas composition was measured using an HP 6890 gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with a thermal conductivity detector and a 5 m x 2.1 mm Carboxen-1000 column (Supelco, Bellafonte, PA) with argon as the carrier gas. Gas flow was measured by bubble counters interfaced with a data acquisition system (Tartakovsky et al., 2008). The pH and conductivity of the effluent were measured using a pH meter (Accumet Excel XL 30, Fisher Scientific, Pittsburgh, PA, USA) and a conductivity meter (Accumet Basic AB 15), respectively.

4.3.3 Media and growth conditions for *Geobacter sulfurreducens*

G. sulfurreducens strain PCA (DSMZ 12127) was obtained from DSMZ (German collection of microorganisms and cell cultures, Braunschweig, Germany) and was grown on minimal media as indicated by DSMZ (DSMZ medium 826). Growth media contained (per liter): 1.5 g NH_4Cl, 0.6 g Na_2HPO_4, 0.1 g KCl and 0.82 g Na-acetate. For media preparation, all the ingredients, except the fumarate, vitamins and $NaHCO_3$, were first dissolved together, the solution was then boiled and cooled down to room temperature while gasing the medium with 80% N_2/20% CO_2. $NaHCO_3$ was then added and 27 mL of this medium were transferred into 60 ml serum bottles under a N_2/CO_2 atmosphere. Serum bottles were

crimped and autoclaved. In parallel, a 16 % (w/v) Na_2-fumarate solution, a 5X - vitamins stock solution (DSMZ medium 141) and a 0.1 M HCl solution were prepared as per the recipe provided by DSMZ, filter-sterilized and degassed at room temperature for 30 min and then subjected to 4 cycles of purging and flushing with Ar to remove any remaining O_2. A 2.5% cysteine sulphide solution was also prepared and then autoclaved. The indicated amounts of each solution were added into the bottles prior to inoculation.

A 10% (vol/vol) inoculum was used, giving a final volume of 30 mL in the serum bottles. 300 µl of a 2.5% Na_2S solution was added in the end to remove traces of oxygen in the headspace. The pH of the medium was adjusted to 6.8-7.0. The bottles were incubated at 30°C. Subsequent inoculation procedures were always performed as above, for each phase, unless indicated.

4.3.4 Bottle tests with *G. sulfurreducens*

Bottle tests were carried out to study *G. sulfurreducens's* growth on acetate and CO. Fumarate was used as the electron acceptor in all tests, unless indicated. The tests were performed under three different concentrations of CO: 20%, 50% and 100% of the headspace. For control experiments, *G. sulfurreducens* was grown in a 100% N_2 atmosphere, in the presence of acetate, at a pH of 7.0. This culture was used as an inoculum for *G. sulfurredencens's* growth test on acetate + 20% CO. The culture from these bottles was subsequently used as an inoculum to test growth on 50% CO + acetate + fumarate, 20% CO + fumarate and 20% CO only. The cultures grown in 50% CO + acetate + fumarate were used as inoculum for 100% CO + acetate + fumarate tests. Gas samples were taken only at

the beginning and at the end of the tests to avoid contamination with oxygen during the tests. For tests on 20% CO + fumarate and 20% CO only, modified minimal media lacking Na-acetate was used. All the tests were performed in triplicates.

4.3.5 DNA extraction and PCR amplification of 16S rDNA sequences

Both MFCs (MFC1 & MFC2) were dismantled, and the anodes were removed from the anodic compartment in a sterile fume hood. Pieces of carbon felt (1 cm x 1 cm x 5 cm) were cut from the middle and bottom of each anode using a sterile surgical scissor and stored at -20°C until use. Total genomic DNA was extracted from the anode samples using the PowerSoil^{TM} DNA isolation kit (MOBIO Laboratories, Carlsbad, USA) according to the instructions of the manufacturer. The extracted DNA was then used as a template to amplify the 16s rDNA region of the eubacterial and archaeal populations by PCR, using universal primers GC-341f (5'-CCT ACG GGA GGC AGC AG-3') and 758r (5'-CTA CCA GGG TAT CTA ATCC-3') for eubacteria and primers GC-931f (5'-AGG AAT TGG CGG GGG AGCA- 3') and 1392r (5'-ACG GGC GGT GTG TGC- 3') for archaea (Tartakovsky et al., 2001). The sequence of the GC clamp added to the forward primers was: CGCCCGCCGCGCGCGGGGGGCGGGGCGGGGGCACGGGGGG.

PCR amplifications were performed in a PCR thermal cycler (Eppendorf Mastercycler Pro. Missisauga, Canada), in a final volume of 30 µL containing 15 µl of TAQ green master mix, 0.5 µM of each primer, 1 ng μL^{-1} of genomic DNA and sterile Millipure water. Amplification conditions were as follows: an initial denaturation step of 5 min at 94 °C, then 20 cycles of denaturation (30 s at 94 °C), annealing (30 s at 55 °C), and elongation (30 s at

72 °C). A final elongation step was of 10 min at 72 °C. PCR products were verified by electrophoresis on a 1.4% agarose gel followed by visualization with UV illumination after Sybr Safe staining (Invitrogen, Carlsbad, CA, USA).

4.3.6 DGGE and 16S rDNA sequencing

DGGE of PCR products were performed with a DCode Gene^{TM} System (Bio-Rad, Hercules, CA, USA). PCR samples were concentrated and 400 ng were loaded onto a 40% to 60% urea-formamide denaturant gradient gel (8% (wt/vol) polyacrylamide in TAE (40 mM Tris-acetate pH 7.4, 20 mM acetate, 1mM Na_2EDTA). Electrophoresis was performed in TAE buffer at a temperature of 60°C and at a constant voltage of 80 V for 16 hours. After electrophoresis, the gel was stained for 30 min with Vistra Green (Molecular Dynamics, CA, USA). Densitometric scanning of fluorescent DNA fragments was performed with the Molecular FluorImager (Molecular Dynamics, Sunnyvale, CA, USA) and results were analyzed using ImageQuaNT software (Molecular Dynamics). Bands of interest were excised from the gel, and DNA was eluted in milli Q water and reamplified by PCR as described above. PCR products were then purified using the QIAquick purification kit (Qiagen, Valencia, CA, USA) and sequenced at the Université Laval (Québec, QC, Canada). Sequences were compared to those in the GenBank database using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI) to determine the phylogenetic affiliations.

4.4 Results and Discussion

4.4.1 Microbial community analysis

Diverse microbial communities were found to be present at the anodes of both CO-fed MFCs. The identified microorganisms belonged to the genera *Geobacter*, *Desulfovibrio*, *Clostridium*, and *Ruminococcus* for eubacteria and to the genera *Methanobacterium*, *Methanofollis* and *Methanosaeta* for archaea. In addition, a number of uncultured bacteria and archaea were identified.

Eubacterial community

The eubacterial populations identified at the anodes primarily consisted of sulfur reducing bacteria (Table 4.1), belonging to the family *Geobacteraceae* and *Desulfovibrioceae.* Sulfur reducers are known to be nutritionally versatile and have already been identified in MFCs fed with different substrates (Logan, 2008). *G. sulfurreducen*s was found to be present at the anodes of both MFCs. However, this microorganism had not been detected when DGGE analyses were performed on the initial sludge used as inoculum for both MFCs (data not shown). As relatively less abundant species could be missed on DGGE patterns (Ercolini, 2004), this suggests that *G. sulffurreducens* was plausibly present at non-detectable levels in the initial sludge and the MFC operating conditions have stimulated its growth at the anode, thereby leading to its identification. The ability of this microorganism to use H_2 and acetate for electricity generation in an MFC has been previously demonstrated (Bond & Lovley, 2003). However, the ability of this microorganism to grow in the presence of CO or use CO as an electron donor has not been demonstrated yet.

Table 4.1 Eubacteria populations identified in MFC1 and MFC2.

Identified Microorganism	Identity (%)	Origin/ Growth Conditions	Hypothesized Activity in a CO-fed MFC	MFC	Reference
Geobacter sulfurreducens	98	Acetate, H_2/CO_2	Electricigenesis	1,2	(Bond & Lovley, 2003)
Uncultured *Geobacter sp.*	98	Isolated from Municipal waste water plant	Electricigenesis	1	*HM991634
Geobacter sp. Lac 319	98	Isolated from the microbial community enriched at the Titanium based anode of a MFC	Electricigenesis	1	(Michaelidou et al., 2011)
Uncultured bacterium	98	Isolated from the microbial community enriched at the anode of waste water fed MFC	-	1	*GU903481
Uncultured bacterium	98	Isolated from the microbial community present at the anode of an MFC fed with different electron donors	-	1	(Jung & Regan, 2007)
Uncultured bacterium	98	Isolated from an MFC fed with waste water	-	1	(Lefebvre et al., 2010)
Uncultured bacterium	98	Isolated from an upflow MFC anode	-	1	*EF515438
Desulfovibrio gigas	99	H_2/CO_2	Electricigenesis	1,2	(Brandis & Thauer, 1981)
Desulfovibrio paquesii	99	H_2/CO_2	Electricigenesis	1,2	(Houten et al., 2009)
Clostridium sticklandii	99	CO, CO_2	Acetogenesis	2	(Fonknechten et al., 2010)
Ruminococcus hydrogenotrophicus	97	H_2/CO_2	Homoacetogenesis	2	(Bernalier et al., 1996)

*GenBank accession no.

Two metalloenzymes, the CO dehydrogenase (CODH), and the acetyl-CoA synthase (ACS) are fundamental for growth on CO. CODH oxidizes CO to CO_2 or alternatively reduces CO_2 to CO. These reactions when coupled to ACS, form a mechanism for generating Acetyl-CoA for cell carbon synthesis (Ragsdale, 2004). The complete genome of *G. sulfurreducens* has been sequenced and was found to contain CODH–related genes and a Wood-Ljungdahl gene cluster (Methe et al., 2003). The presence of these genes indicated that *G. sulfurreducens* might be able to use exogenous CO or at least might be tolerant to CO.

This hypothesis was tested in bottle tests, in which a pure culture of *G. sulfurreducens* strain PCA was grown under different concentrations of CO and acetate (Table 4.2). The results showed that *G. sulfurreducens* could grow under 20%, 50% and even 100% CO in the presence of acetate and hence, demonstrated its tolerance to CO. The bacterium could not grow solely on CO, however, it could convert CO into CO_2, which was evidenced by a decrease in CO and an increase in CO_2 concentrations (Table 4.2).

Desulfovibrio species such as *D. gigas,* and *D. paquesii,* were detected in both MFCs. Alike *G. sulfurreducens*, *D. gigas,* and *D. paquesii* had not been detected when DGGE analyses were performed on the initial sludge used as inoculums for both MFCs (data not shown), again suggesting that the MFC operating conditions might have simulated their growth at the anode. *Desulfovibrio* species, have already been identified in bioreactors fed with syngas and have also been found to be present in the anodic microbial communities of a MFC fed with CO saturated wastewater, suggesting CO tolerance by at least some members of this species (Houten et al., 2006; Lee et al., 2003). The ability to utilize CO as the sole source of electrons for sulfate and metal reduction by some of its members has also

been demonstrated (Parshina et al., 2010). Such species were hypothesized to be responsible for CO conversion into CO_2 and H_2 in the study of Preez and Maree (Preez & Maree, 1994), where producer gas was used as an energy source for biological sulfate and nitrate removal. *D. paquesii* and *D. gigas* which were identified in our study are capable of using H_2 as an electron donor for sulfate reduction (Brandis & Thauer, 1981; Houten et al., 2009). *D. paquesii* has already been isolated in a reactor fed with syngas and has been suggested to be tolerant to CO (Brandis & Thauer, 1981; Houten et al., 2009). To our knowledge, the tolerance of *D. gigas* to CO has not been tested. However, its identification in our reactor suggests that it might be tolerant to CO. Further experiments, such as bottle tests with CO, would be required to test the ability of *D. gigas* to oxidize CO.

Community analysis also led to the identification of the acetogens R. *hydrogenotrophicus* and *C. sticklandii*. These acetogens had not been detected during the DGGE analyses of the initial inoculum sludge. *R. hydrogenotrophicus* is known to form acetate by acetogenic hydrogenation (Bernalier et al., 1996) where H_2 and CO_2 are converted to acetate according to the equation:

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad \text{(4.1)}$$

Homoacetogens such as *R. Hydrogenotrophicus* have been widely isolated in syngas-fed bioreactors, forming acetate from CO_2 and H_2, produced by the conversion of CO by the *Desulfovibrio* species (Lens et al., 2002; Sipma et al., 2006). *C. sticklandii*, might also have the capacity to form acetate from CO as several clostridia are capable of using CO as their sole source of energy while forming acetate and CO_2 (Sipma et al., 2006), according to the equation:

$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$ ..... (4.2)

This hypothesis is strengthened by the presence of gene clusters in the genome of *C. sticklandii*, which encode the complete Wood-Ljungdahl pathway and the glycine synthase reductase pathway, both of which lead to acetate formation (Fonknechten et al., 2010). Although the presence of *C. sticklandii* at the anode of MFC2, suggests its tolerance to CO, further experiments would be required to confirm its growth solely on CO. As acetate was the major metabolite in both MFCs, one could conceive that there were more acetogens present but we were not able to isolate them in the DNA extraction-PCR amplification process.

An important fact to be noticed is that, *Ruminococcus* and *Clostridium* species were not identified at the anode of MFC1 (Table 4.1). This difference in the anodic eubacterial populations between MFC1 and MFC2 might be due to higher substrate availability in MFC2. Due to the utilization of silicone membrane systems for gas delivery in MFC2, the gas to liquid mass transfer was improved (Hussain et al., 2011a), which could have stimulated the growth of these species. Conceivably, these species were present in MFC1, but were plausibly not detected in DGGE analyses due their less abundance in the system.

Table 4.2 G. *sulfurreducens* growth on CO and acetate.

Medium	**OD-600 nm** Initial / Final	**Acetate (mg L^{-1})** Initial / Final	**CO (%)** Initial / Final	**CO_2 (%)** Initial / Final
20 % CO + acetate + fumarate	0.05 ± 0.002/ 0.39 ± 0.1	664 ± 32.1 / 35 ± 26.9	20 ± 1.7 / 14 ± 0.6	7.4 ± 1.2 / 17 ± 1.4
50% CO + acetate + fumarate	0.06 ± 0.002 / 0.34 ± 0.03	264 ± 10/ 0	43 ± 4.3 / 26 ± 2.7	10 ± 0.1 / 23 ± 0.1
100 % CO + acetate + fumarate	0.04 ± 0.005/ 0.33 ± 0.01	664 ± 29 / 45 ± 38.8	96 ± 1.1 / 79 ± 1	1.6 ± 0.2 / 13 ± 0.6
20 % CO	0.04 ± 0.04/ 0.03 ± 0.01	0	19 ± 1.9 / 10 ± 4	8 ± 0.7 / 15 ± 1.1
20 % CO + fumarate	0.01 ± 0.002/ 0.03 ± 0.01	0	20 ± 2.1 / 10 ± 1.3	11 ± 0.4 / 17 ± 1.5

Archaeal community

The archaea identified in this study were all methanogens (Table 4.3) and primarily possessed the hydrogenotrophic pathway, in which CO_2 is sequentially reduced to CH_4, with H_2 serving as the electron donor, according to the equation:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad \text{.....} \quad (4.3)$$

The H_2 production from CO (water gas-shift reaction) in our CO-fed MFCs might have stimulated the growth of these methanogens. Hydrogenotrophic methanogens have already been reported to be the dominant methanogens in syngas-fed bioreactors, due to i) lower threshold for H_2 as compared with that of the acetogens and ii) the energy yield from the conversion of CO_2 and H_2 to CH_4, which is greater than the conversion of acetate to CH_4 (Houten et al., 2006).

Methanobacterium species identified in this study (*Methanobacterium* sp. OM15, *M. formicicum, and M. beijingense*) and *Methanofollis liminatans* are known to utilize H_2 and CO_2 for methane formation (Houten et al., 2006; Imachi et al., 2011; Wu et al., 1993). To our knowledge, the CO tolerance of these microorganisms in pure cultures has not been studied yet. However, CO tolerance for some of them has been reported in a mixed culture. *M. formicicum*, *M. liminatans* and *Methanobacterium* sp. OM15 were notably identified in a syngas-fed reactor treating sulfate and metal-rich waste water (Houten et al., 2006), suggesting their tolerance to CO. The CO tolerance of *M. formicicum* was also demonstrated in a mixed culture with *R. rubrum*, where *R. rubrum* performed the water gas shift reaction to form H_2 and CO_2, which was subsequently converted to CH_4 by *M. formicicum* (Daniels et al., 1977).

Table 4.3 Archaea populations identified in MFC1 and MFC2.

Identified Microorganism	Idenitity (%)	Origin/ Growth Conditions	Hypothesized Activity in a CO-fed MFC	MFC	Reference
Uncultured Archaeon	99	Isolated from a bioreactor treating Waste water	-	1,2	(Tsushima et al., 2010)
Uncultured Archaeon	99	Isolated from a bioreactor treating Waste water	-	1	(Tsushima et al., 2010)
Methanobacterium sp. T01,C5/51	99	Rumen Methanogens	-	1	*AB288275, AJ550159
Methanobacterium sp.OM15,MO-MB1	99	H_2/CO_2	Hydrogenotrophic Methanogenesis	1	(Imachi et al., 2011)
Methanosaeta concilii	99	Acetate	Acetoclastic Methanogenesis	1,2	(Huser et al., 1982)
Methanobacterium formicicum	99	H_2/CO_2, Formate	Hydrogenotrophic Methanogenesis	1	(Wu et al., 1993)
Uncultured Archaeon	99	Isolated from an anaerobic digester	-	1,2	*AB494254
Uncultured Archaeon	99	Isolated from a bioreactor fed with Waste water	-	1,2	(Tsushima et al., 2010)
Methanobacterium beijingense	99	H_2/CO_2,formate	Hydrogenotrophic Methanogenesis	1	(Ma et al., 2005)
Uncultured Archaeon	98	Isolated from an anaerobic digester	-	1	(Ueki et al., 2009)
Uncultured Archaeon	99	Methanogenic digester	-	1	(Kovacik et al., 2010)
Uncultured Archaeon	99	Anaerobic digester treating waste water	-	1	(Godon et al., 1997)
Uncultured Archaeon	99	Waste water treatment plant	-	1	*CU466582
Uncultured Archaeon	99	Isolated from landfill leachate	-	1	(Huang et al., 2003)
Methanofollis liminatans	98	H_2/CO_2,Formate	Hydrogenotrophic Methanogenesis	2	(Zellner et al., 1999)
Methanobacterium sp.MO-MB1	96	H_2/CO_2	Hydrogenotrophic Methanogenesis	2	(Imachi et al., 2011)
Methanobacterium petrolearium	96	H_2/CO_2	Hydrogenotrophic Methanogenesis	2	(Mori and Harayama, 2011)

*GenBank accession no.

In addition to hydrogenotrophic methanogens, the acetoclastic methanogen, *Methanothrix soehngenii* (now called *Methanosaeta concilii*) (Huser et al., 1982; Wayne, 1994) was also identified. Acetoclastic methanogens in the presence of acetate generate metabolic energy by interfacing the Wood-Ljungdahl pathway to the pathway of methanogenesis. *M. concilii* uses acetate as a sole source of energy to form CH_4 and CO_2, as per the equation:

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad \text{.....} \quad (4.4)$$

Although they possess the enzyme CODH, such methanogens do not convert CO to acetyl-CoA. It has been suggested that CO might be involved as a CODH/ACS-bound intermediate in acetoclastic energy metabolism among methanogens such as *M. concilii* (Oelgeschlager & Rother, 2008). The exogenous CO may form an unidentified bound intermediate during the conversion of the carboxyl group of the acetate to CO_2 (Daniels et al., 1977). Therefore, it could be hypothesized that the exogenous CO in both our MFCs was oxidized by *M. concilii* into CO_2 to derive electrons for the reduction of methyl-S-CoM into methane, as per the combined equation:

$$CO + H_2O + CH_3\text{-S-CoM} \rightarrow CH_4 + CO_2 + \text{HS-CoM} \quad \text{.....} \quad (4.5)$$

4.4.2 Pathways for electricity generation in a CO-fed MFC

Following the microbial community analysis, pathways involved in electricity generation in CO-fed MFCs can be better elucidated and understood. Acetate formation from CO, followed by acetate oxidation to produce electricity by CO tolerant electricigenic microorganisms has been previously suggested to be one of the prominent pathways for electricity generation (Mehta et al., 2010). This pathway can now be conceptualized based

upon the identification of acetogens such as *C. sticklandii*, which might have the capacity to form acetate from CO, followed by the demonstrated CO-tolerance of the electricigenic *G. sulfurreducens*, whose ability to produce electricity in an acetate-fed MFC has been previously reported (Bond & Lovley, 2003). The following stoichiometric equations describe this pathway:

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \quad \text{.....} \quad (4.6)$$

$$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+ \text{.....} \quad (4.7)$$

The presence of H_2 in the off-gas also raised the possibility of electricity generation via hydrogen as previously hypothesized (Mehta et al., 2010). H_2 could have been formed from CO by the members of the *Desulfovibrio* species by the following reaction, as previously described (Sipma et al., 2006):

$$CO + H_2O \rightarrow H_2 + CO_2 \text{.....} \quad (4.8)$$

The H_2 produced could then be utilized by *G. sulfurreducens* for electricity generation, as previously proposed (Hussain et al., 2011b). The ability of a microorganism to produce electricity in an MFC could include any microorganism capable of extracellular electron transfer, even if its capacity has not yet been experimentally evidenced (Logan, 2009). *D. gigas* identified in this study, has been reported to reduce Tc(VII) utilizing H_2 as the electron donor (Lovley, 1993). Therefore, it could be suggested that the *Desulfovibrio* species identified at the anode of MFC2 in this study, might be involved in electricigenesis using H_2.

Substantial amounts of acetate were previously reported to be present in the effluent, when the MFCs were fed with H_2/CO_2 (Hussain et al., 2011b; Mehta et al., 2010). This

observation can now be related to the presence of homoacetogens such as *R. hydrogenotrophicus*, identified in this study. Their presence also demonstrates that electricity generation in CO-fed MFCs primarily proceeds via acetate conversion to electricity.

Significant amounts of CH_4 were present in the off-gas samples of both MFCs, which was previsously hypothesized to be responsible for the low Coulombic efficiency (CE) of the systems (Hussain et al., 2011a; Mehta et al., 2010). The hypothesis can now be related to the presence of a large number of hydrogenotrophic and acetogenic methanogens at the anode, which must have competed with the eletricigenic bacteria in the MFCs for the electron donor, resulting in a low CE. A similar observation was also made in the study of Kim et al. (2005), where a 30 % increase in CE was observed when a methanogenic inhibitor was utilized.

Overall this study presents the microbial diversity prevalent at the anodes of CO-fed MFCs. The microbial community analysis provided a better understanding of the range of reactions catalyzed by microorganisms in CO-fed MFCs and helped elucidate the pathways involved in electricity generation. *G. sulfurreducens*, along with other sulfate reducers were found to be present in both MFCs. Bottle tests demonstrated the ability of *G. sulfurreducens* to grow in a 100% CO environment, in the presence of acetate. Based upon the current literature and findings of this study, they might be the primary microorganisms responsible for electricity generation in a CO-fed MFC. The results presented herein, may lead to the development of CO-fed MFCs with engineered consortia. A CO-fed MFC seeded with pure cultures of electricigenic microorganism such as *G. sulfurreducens* in a syntrophic

relationship with carboxydotrophic microorganism might be capable of achieving high power densities, due to the elimination of alternative metabolisms such as methanogenesis, which competes with the electricigenic microorganisms for the electron donor and use electron acceptors which do not result in electricity generation, resulting in low coulombic efficiency. Acetate-fed MFCs seeded with a pure culture of *G. sulfurreducens* were recently reported to achieve power densities that were comparable to the MFCs inoculated with an anaerobic sludge (Nevin et al., 2008).

4.5 Conclusion

Diverse microbial communities were found to be present at the anode of CO-fed MFCs. The identified archaea populations primarily consisted of hydrogenotrophic methanogens, while the eubacterial populations were dominated by the members belonging to *Geobacteraceae* and *Desulfovibrioceae*. The study demonstrates the ability of *G. sulfurreducens* to grow in a 100% CO environment, in the presence of acetate and discusses the underlying role played by sulfur reducing bacteria in electricity generation from CO. The microbial community analysis provided a better understanding of the range of reactions catalyzed by microorganisms in CO-fed MFCs and helped elucidate the pathways involved in electricity generation. The identification of large number of methanogens suggests that a part of the anodic microbial community is sustained by alternative metabolism and competes with the electricigenic microorganisms for the electron donor and use electron acceptors which do not result in electricity generation, resulting in low Coulombic efficiency.

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Connecting text

Chapter 3 demonstrated improved MFC performance under mesophilic conditions by increasing the CO gas-liquid mass transfer. Subsequently in chapter 4 the microbial communities and biotransformation pathways involved in electricity production from CO/syngas in a mesophilic MFC were elucidated.

Considering that syngas is primarily a hot gas with temperatures of 40-55 °C at the exit of the gasification process, the operation of CO/syngas-fed MFCs at thermophilic temperatures is of interest. However, thermophilic operations of CO/syngas-fed MFCs poses several challenges including reduced CO/syngas solubility in the anodic liquid, increased anodic liquid evaporation and lack of known thermophilic CO-tolerant electricigenic microorganisms.

The following study demonstrated electricity production in a thermophilic MFC fed with syngas, and elucidated the microbial communities and biotransformation pathways involved in electricity production at elevated temperatures. The study was published in *Journal of Enzyme and Microbial Technology* (2012), 51; 163-170.

CHAPTER 5

The performance of a thermophilic microbial fuel cell fed with synthesis gas

5.1 Abstract

This study demonstrated electricity generation in a thermophilic microbial fuel cell (MFC) operated on synthesis gas (syngas) as the sole electron donor. At 50 ºC, a volumetric power output of 30-35 $mW\,L_R^{-1}$ and syngas conversion efficiency in the range of 87-98 % was achieved. The observed pathway of syngas conversion to electricity primarily consisted of a two-step process, where the carbon monoxide and hydrogen were first converted to acetate, which was then consumed by the electricigenic bacteria to produce electricity. A denaturing gradient gel electrophoresis (DGGE) analysis of the 16S rDNA revealed the presence of *Geobacter* species, A*cetobacter*, methanogens and several uncultured bacteria and archaea in the anodic chamber.

Keywords: MFC; synthesis gas; carbon monoxide; thermophilic conditions

5.2 Introduction

Electricity production from diluted organic matter in mesophilic and thermophilic microbial fuel cells (MFC) has been extensively demonstrated (Logan, 2010; Logan & Regan, 2006; Lovley, 2008). However, solid organic wastes (e.g. organic fraction of municipal solid wastes, agricultural wastes such as straw, corn husks, etc.) might be pre-treated and diluted to be used as an MFC fuel. To avoid excessive water and energy consumption, such wastes are often transformed in a gasification process to synthesis gas (syngas), which mainly consists of carbon monoxide (CO) and hydrogen (Munasinghe & Khanal, 2010a; Perry et al., 1997). Recently, electricity generation from CO and syngas in a mesophilic MFC has been demonstrated, thus further expanding the range of MFC applications (Kim & Chang, 2009; Mehta et al., 2010).

CO and syngas-fed MFCs have been so far operated at mesophilic temperatures. Considering that at the exit of the gasification process syngas temperatures could be in a range of 45–55 ºC, the operation of the MFC at thermophilic temperatures might be preferable as it eliminates the need for further syngas cooling and might lead to a higher biocatalytic activity (Jong et al., 2006; Mathis et al., 2008). The thermophilic conditions would also lead to reduced oxygen solubility, which is beneficial considering that even trace amounts of unreacted O_2 diffusing through the cathode can inhibit the anaerobic carboxydotrophic microorganisms which are highly sensitive to the presence of O_2 (Davidova et al., 1994). However, syngas conversion to electricity in an MFC involves a combination of microbiological, electrochemical and transport phenomena, all of which are affected by temperature, such that the overall outcome of its increase is uncertain.

This study presents the first attempt to operate a syngas-fed MFC at a moderately thermophilic temperature of 50 °C. The analysis of the off-gas and effluent composition is utilized to propose the pathways leading to electricity generation under thermophilic conditions. To confirm the proposed pathways, the observed metabolites were compared to the microbial communities identified in the anodic chamber by the DGGE of the polymerase chain reaction (PCR) amplified 16S rDNA fragments.

5.3 Materials and Methods

5.3.1 Medium composition and analytical methods

The composition of the stock solution of nutrients was (in g L^{-1}): 1.87 NH_4Cl, 14.81 KCl, 6.40 K_2HPO_4, 4.07 KH_2PO_4 and 0.215 yeast extract. The trace metal stock solution was prepared according to Tartakovsky et al. (2008), and contained (in g/L): 2 $FeCl_2$4H_2O, 0.05 H_3BO_3, 50 $ZnCl_2$, 0.03 $CuCl_2$, 0.5 $MnCl_2$4H2O, 0.5 (NH_4)6Mo7$O_2$44H2O, 0.5 $AlCl_3$, 0.5 $CoCl_2$6H_2O, 0.5 $NiCl_2$, 0.5 EDTA, and concentrated HCl (1 mL). The chemicals used were of analytical grade. The stock solutions were filter - sterilized with a 0.22 μm filter (Fisher Scientific, Ottawa, ON, Canada) and maintained at 4°C until use. The influent solution was prepared by adding 35 mL of the nutrient solution and 1 mL of the trace metal solution to 1 L of de-ionized water. This solution had a conductivity of 12 -14 mS cm^{-1}.

Acetate and other volatile fatty acids (VFAs) were analyzed using an Agilent 6890 gas chromatograph (Agilent Technologies Inc, Santa Clara, CA, USA) equipped with a flame ionization detector. The off-gas flow rate was measured using the MilliGascounter™ (Ritter Apparatus, Bochum, Germany). The gas composition was measured using an HP 6890 gas

chromatograph (Hewlett Packard, Palo Alto, CA) equipped with a thermal conductivity detector and a 5 m x 2.1 mm Carboxen-1000 column (Supelco, Bellafonte, PA) with argon as the carrier gas. Further details of the analytical methods are provided in Guiot et al. (2011).

5.3.2 Inoculum

The thermophilic mixed culture capable of growth on CO was obtained from a mesophilic anaerobic granular sludge treating agricultural wastes (Lassonde Inc., Rougemont, QC, Canada). The following enrichment procedure was used. A 1 L serum bottle containing 200 mL of the mesophilic sludge and 300 mL of liquid medium (same as the influent solution) was incubated for one week under 100% CO atmosphere in a rotary shaker incubator at 100 rpm and 45 °C. The temperature was then raised to 50 °C. The CO concentration in the headspace of the bottle and the VFA composition of the liquid were analyzed weekly. The headspace was replenished with 100% CO every 2-3 days. The bottle was maintained at 50 °C for one month and then used as the inoculum.

5.3.3 MFC design, operation, and characterization

The MFC experimental set-up was similar to that previously used for the demonstration of electricity production from CO in a mesophilic MFC equipped with silicone tubing for CO supply (Hussain et al., 2011). The 50 mL anodic compartment contained a 10 cm x 5 cm carbon felt anode (SGL Canada, Kitchener, ON, Canada), with a volume of 25 mL. A CO/FeTMPP cathode attached to the side of the anode compartment was separated from the anode by a non-conductive 1mm synthetic cloth. The cathode was prepared as described in

Neburchilov et al. (2011). Syngas was supplied to the anodic liquid through silicone tubing coiled around a second piece of carbon felt separated from the anode (Fig. 5.1A) and thereby, excluded from the electrical circuit by the non-conductive synthetic cloth. The silicone tubing (VWR International LLC, Radnor, PA, USA) had a wall thickness of 90 μm, an external diameter of 1.76 mm, and a total surface area of 50 cm^2. The inlet of the silicone tubing was connected to the gas line, while the outlet was connected to a manometer. The solution of nutrients and microelements was fed in at a rate of 10 mL d^{-1} using a peristaltic pump. The schematic diagram of the experimental setup is shown in Figure 5.1B.

The anodic liquid was maintained at 50 °C by heating the liquid in the external recirculation loop of the MFC. To simulate syngas feeding, CO and H_2 (50:50 v/v) were simultaneously fed at desired flow rates. During the start-up, the MFC was operated with a constant external resistance of 500 Ω. Once a steady power output was observed, the external resistance was optimized using the Perturbation and Observation MPPT algorithm, which constantly adjusted the external resistance to maximize the MFC power output in real time (Woodward et al., 2009).

Syngas flow rates of 4, 6, 8 and 10 L L_R^{-1} d^{-1} (expressed as L of syngas fed per L of anodic chamber volume per day) were tested. Each flow rate was tested for a period of at least 5 days. In addition to MFC operation on syngas, several single electron donor tests were carried out. In the hydrogen test, a H_2/CO_2 (80:20 v/v) mixture was fed at a rate of 6 L L_R^{-1} d^{-1}. The carbon monoxide test used a CO/N_2 (50:50 v/v) gas mixture also fed at a flow rate of 6 L L_R^{-1} d^{-1}. Each test was performed for 3 days.

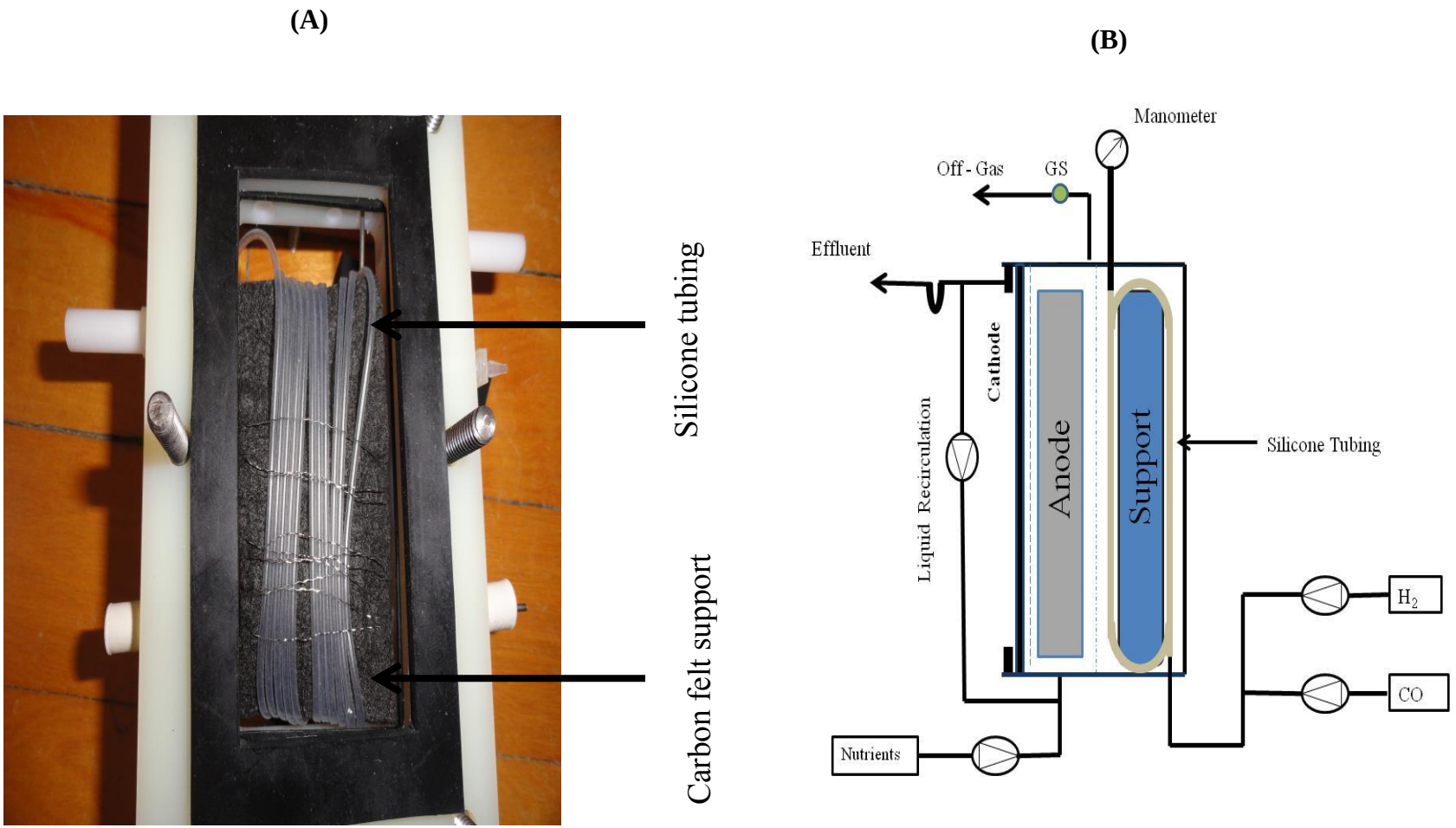

Figure 5.1 (A) Silicone tubing utilized for syngas delivery (B) Diagram of the experimental set-up of syngas-fed MFC, where GS represents the gas meter.

The MFC voltage was measured and recorded at 20 min intervals using Labjack U12 (Labjack Corp., Lakewood, CO, USA). Polarization tests were carried out at the end of each test following the procedure described in (Logan, 2008; Mehta et al., 2010).

The Coulombic efficiency (CE) of the syngas-fed MFC was calculated as:

$$CE = \frac{I\Delta t}{Fn\left(\frac{M_{CO,in} - M_{CO,out}}{M_{CO}} + \frac{M_{H2,in} - M_{H2,out}}{M_{H2}}\right)} 100\% \quad \text{..... (5.1)}$$

Where, I is the average current (A), Δt is the time interval during which the current was measured (s), M_{CO} is the molar mass of CO (g/mol), M_{H2} is the molar mass of H_2 (g/mol), F is the Faraday constant (C/mol-e^-), n is the number of electrons exchanged (mol-e^-/mol), $M_{CO,in}$ and $M_{H2,in}$ are the amounts of CO and H_2, respectively, supplied to the MFC during Δt (g), $M_{CO,out}$ and $M_{H2,out}$ are the amounts of CO and H_2, respectively, recovered (g) in the off-gas during Δt.

5.3.4 K_La measurements and CO conversion tests

To estimate the volumetric mass transfer coefficient (K_La) and to study the metabolic products of CO consumption in the absence of electricigenic activity, a reactor similar in design to the MFC was used. Modifications were required in order to avoid air diffusion through the cathode surface. Accordingly, a reactor was built similar to the MFC with the liquid compartment containing two pieces of carbon felt (10 cm x 5 cm x 0.5 cm) with the silicone tubing coiled around one of the felts. The cathode was replaced with a solid nylon wall. The reactor had an external recirculation loop to provide liquid mixing and heating and was operated under the same conditions as the MFC.

K_La was estimated at 50 °C under abiotic conditions at several CO and syngas flow rates using the method described by Munasinghe et al. (2010b), by introducing a step change in the gas supply rate and then evaluating the liquid phase concentration as a function of time. The dissolved CO concentration was determined as described in Lide and Frederikse (1995). The K_La values were estimated based on the analytical solution of the mass balance equation:

$$\ln\left(\frac{C_{ct}-C_0}{C_{ct}-C_t}\right)=(K_La)t \quad \text{..... (5.2)}$$

Where: C_o is the initial dissolved CO concentration, C_{ct} is the gas-liquid equilibrium concentration and C_t is the dissolved CO concentration at time t. The slope of the line defined by Eq (5.1) corresponds to the volumetric mass transfer coefficient, K_La (Munasinghe & Khanal, 2010b; Riggs & Heindel, 2006).

The tests of CO bioconversion were performed in the same reactor after its inoculation with 10 mL of the thermophilic enrichment culture. A period of 4 days was allowed to pass before the start of the test for the stabilization of the effluent and off-gas composition. For the metabolite study the reactor was fed with CO at a rate of 4 L L_R^{-1} d^{-1}.

5.3.5 DNA extraction, PCR amplification of 16S rDNA, Denaturing gradient gel electrophoresis (DGGE) and 16S sequencing

After the completion of the MFC tests, total genomic DNA was extracted using the PowerSoil™ DNA isolation kit (MOBIO Laboratories, Carlsbad, USA) as outlined in the instructions provided by the manufacturer. The samples taken from the anode, the carbon felt used to support the silicone tubing, and the anodic liquid samples were used for the analysis.

The extracted DNA was then used as a template to amplify the 16S rDNA region of the eubacterial and archaeal populations by PCR. Universal eubacterial primers GC-341f (5'-CCT ACG GGA GGC AGC AG-3') and 758r (5'-CTA CCA GGG TAT CTA ATCC-3') were used for eubacteria and primers GC-931f (5'-AGG AAT TGG CGG GGG AGCA- 3') and 1392r (5'-ACG GGC GGT GTG TGC- 3') were used for archaea. The GC clamp added to the forward primers had the following sequence: CGCCCGCCGCGCGCGGGGGGCGGGGCGGGGGCACGGGGGG.

PCR amplifications were performed with a PCR thermal cycler (Eppendorf Mastercycler Pro. Missisauga, Canada), in a final volume of 30 µL containing 15 µl of TAQ green master mix, 1.5 µl of each primer (final concentration of 0.5 µM of each primer), 1 ng μL^{-1} of genomic DNA and 9 µl of sterile Millipure water. Amplification conditions were as follows: an initial denaturation step of 5 min at 94 °C, then 20 cycles of denaturation (30 s at 94°C), annealing (30 s at 55 °C), and elongation (30 s at 72 °C). A final elongation step was 10 min at 72 °C. PCR products were verified by electrophoresis on a 1.4% agarose gel followed by visualization with UV illumination after Sybr Safe staining (Invitrogen, Carlsbad, CA, USA).

DGGE analysis of PCR products were performed with a DCode Gene^{TM} System (Bio-Rad, Hercules, CA, USA). PCR samples were concentrated and 400 ng were loaded onto a 40% to 60% urea-formamide denaturant gradient gel (8% (wt/vol) polyacrylamide in TAE (40 mM Tris-acetate pH 7.4, 20 mM acetate, 1mM Na_2EDTA). Electrophoresis was performed in 1X TAE solution (40 mM Tris-acetate pH 7.4, 20 mM acetate, 1mM Na_2EDTA) at a temperature of 60°C and at a constant voltage of 80 V for 16 hours. After electrophoresis, the gel was stained for 30 min with Vistra Green (Molecular Dynamics, CA,

USA). Densitometric scanning of fluorescent DNA fragments was performed with the Molecular FluorImager (Molecular Dynamics, Sunnyvale, CA, USA) and results were analyzed using ImageQuaNT software (Molecular Dynamics). Bands of interest were excised, and DNA was eluted in milli Q water and reamplified by PCR as described above. PCR products were then purified using the QIAquick purification kit (Qiagen, Valencia, CA, USA) and sequenced at the Laval University (Quebec City, QC, Canada). The sequences were compared to those in the GenBank database using the Basic Local Alignment Search Tool (BLAST) at the National Center for Biotechnology Information (NCBI) to determine the phylogenetic affiliations.

5.4 Results and Discussion

5.4.1 CO K_La estimation

When a bioreactor is operated on a gaseous substrate such as syngas, the gas transport to liquid often represents the rate limiting step of the entire process. To estimate the syngas transfer rate to the anodic liquid, K_La measurements were carried out under abiotic conditions, i.e. in the absence of syngas consumption by the thermophilic microbial consortium.

The K_La estimations were carried out by measuring the dissolved gas concentrations and estimating the slope of the curve representing the analytical solution of the material balance (Eq. 5.1). The silicone tubing was operated under a dead-end mode, which allowed for an elevated gas pressure in the system, thus increasing the concentration gradient and hence the mass transfer of the gas (Ahmed & Semmens, 1992). Initially, the test chamber was fed with syngas containing 50 % CO and 50 % H_2. While the dissolved CO

concentration could be measured, measurement of dissolved H_2 was unsuccessful due to its low solubility in water and the insufficient detection limit of the analytical method. Therefore, only the CO K_La was estimated. Feeding the test chamber with a syngas flow of 6 L L_R^{-1} d^{-1} resulted in a CO K_La of 0.72 h^{-1}. By increasing the syngas flow rate to 10 L L_R^{-1} d^{-1}, the K_La was increased to 0.87 h^{-1}. Additionally, the K_La estimation test was repeated with 100% CO. A K_La of 0.86 h^{-1} was observed at a CO flow rate of 3 L L_R^{-1} d^{-1}. Stepping up the CO flow rate to 6 L L_R^{-1} d^{-1} increased the CO K_La to 1 h^{-1} (Fig. 5.2).

The lower CO K_La values estimated in the syngas test as compared to the CO test might be attributed to the lower partial pressure of CO. Nevertheless, the CO K_La values observed during the syngas test were still comparatively higher than a value of 0.63 h^{-1} obtained in our previous study at a CO flow rate of 6 L L_R^{-1} d^{-1} for the mesophilic MFC operated at 35 °C (Hussain et al., 2011). The solubility of gases decreases as temperature increases, however this phenomenon is counteracted by increased molecular diffusivity of the gas into the liquid phase. In addition, the liquid viscosity and surface tension decrease with increased temperature, resulting in the reduction of the thickness of the stagnant liquid layer at the gas-liquid interface and thereby improving the gas transport (Ferreira et al., 2010). Overall, the estimations showed that K_La was marginally improved at 50 °C, thus confirming that a sufficient amount of carbon monoxide can be transferred for the carboxidotrophic metabolism. While the dissolved H_2 measurements were unsuccessful, we hypothesized that the H_2 transfer rate should be also similar to that under the mesophilic conditions. Also, H_2 production from CO through the water-gas shift reaction was observed in the mesophilic MFC (Hussain et al., 2011; Mehta et al., 2010). Assuming that a similar pathway existed at

thermophilic temperatures, it would provide a source of H_2 for the electricigenic microorganisms capable of direct H_2 consumption (Bond & Lovley, 2003).

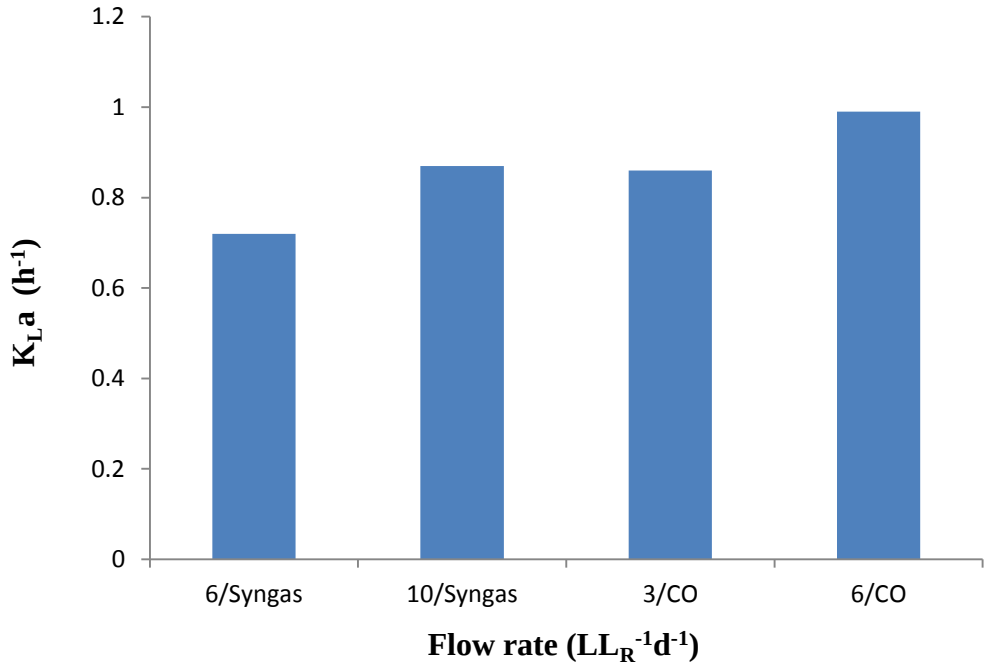

Figure 5.2 Volumetric mass transfer coefficient (K_La) measurements for CO at different syngas and CO flow rates at 50°C. Syngas consisted of 50% CO and 50% H_2 (vol).

5.4.2 MFC operation on syngas

The MFC operation was started with a syngas flow rate of 2 L L_R^{-1} d^{-1}. At the startup the external resistance was set to 500 Ω. To demonstrate the absence of electricity production under abiotic conditions, the MFC was fed with syngas and nutrient solution for 2 days before it was inoculated with the thermophilic enrichment culture. A voltage of 3-5 mV was measured thus indicating a negligible power output under the abiotic conditions. Upon the addition of the thermophilic enrichment culture to the anodic liquid, a steady increase in the

MFC voltage was observed. A voltage of 100 mV, corresponding to a power output of 0.4 $mW\,L_R^{-1}$ was obtained within 4 days of inoculation. At this point, the syngas flow rate was stepped up to 8 $L\,L_R^{-1}\,d^{-1}$. Starting from day 5, the on-line perturbation/observation (P/O) algorithm was used to optimize the external resistance thus maximizing the MFC power output at all times (Woodward et al., 2010). In less than 12 days, the power produced by the thermophilic MFC stabilized at 31 ±0.6 $mW\,L_R^{-1}$. The MFC was operated at this syngas flow rate for 15 days, and then a polarization test was carried out to evaluate the internal resistance (R_{int}), open circuit voltage (OCV), and the maximum power output (P_{max}). An OCV value of 655 mV and a R_{int} value of 49 Ω were estimated based on the polarization curve shown in Fig. 5.3A. Also, a maximum power output (P_{max}) of 33 $mW\,L_R^{-1}$ was obtained, as shown in Fig. 5.3B. The R_{int} estimation agreed well with the optimized R_{ext} chosen by the P/O algorithm, which varied between 45 and 54 Ω under optimized conditions. Following the 8 $L\,L_R^{-1}\,d^{-1}$ test, several syngas flow rates were tested. First, the flow rate was increased to 10 $L\,L_R^{-1}\,d^{-1}$. The increase significantly impeded the MFC performance. The average power output at this syngas flow rate was reduced to 13 $mW\,L_R^{-1}$ (Fig. 5.4) and the syngas transformation efficiency dropped to 73 % as both the CO and H_2 content of the anodic off-gas increased (Table 5.1). The polarization and power curves acquired at the end of the 10 $L\,L_R^{-1}\,d^{-1}$ test affirmed the reduced performance, as P_{max} declined to 16 $mW\,L_R^{-1}$ (Fig. 5.3B) and R_{int} increased to 94 Ω (Table 5.1). Consequently, syngas flow rates higher than 10 $L\,L_R^{-1}\,d^{-1}$ were not tested.

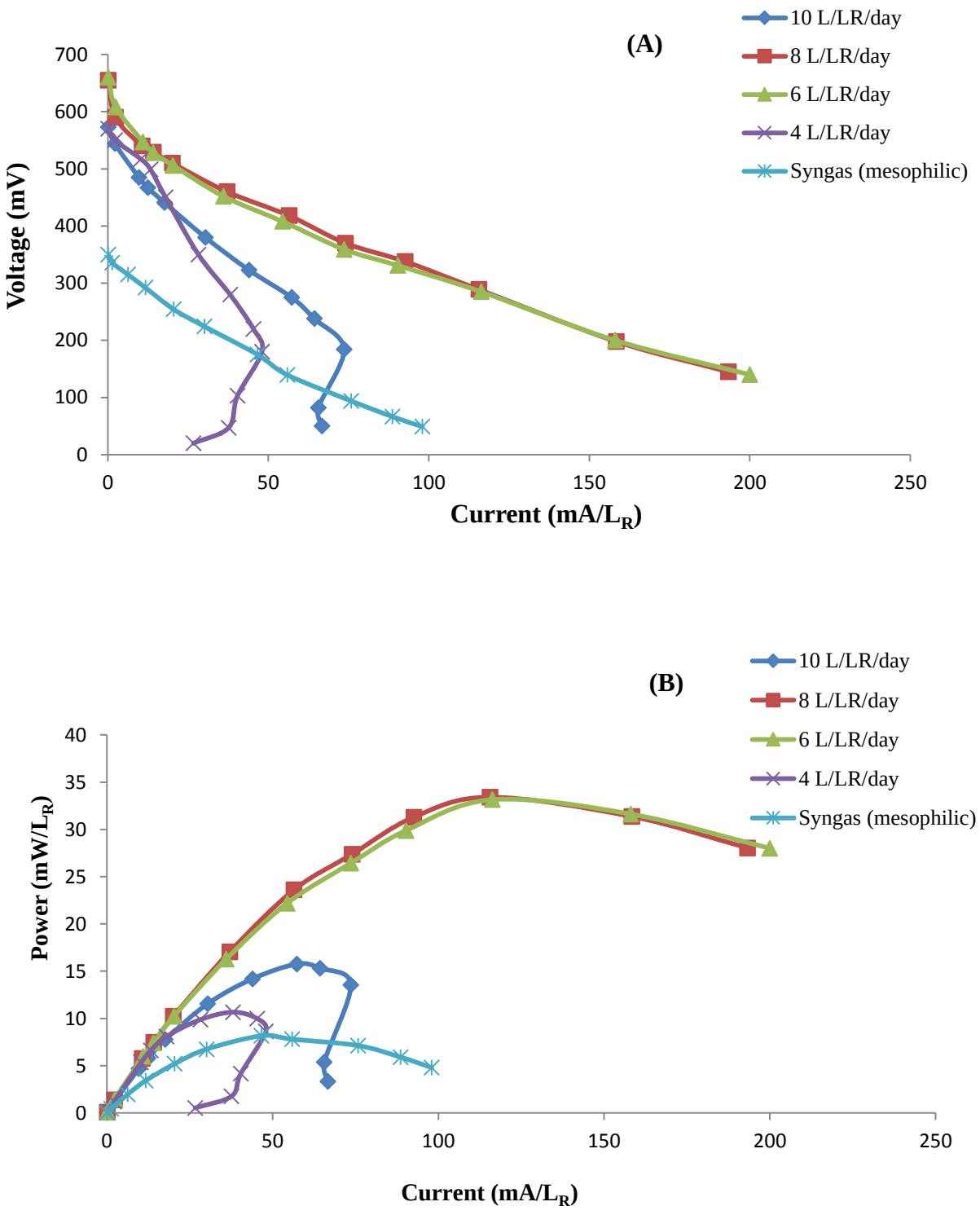

Figure 5.3 (A) Polarization and (B) Power curves for syngas-fed MFC at different syngas flow rates, where syngas (mesophilic) represents the values obtained for a syngas-fed MFC operated at 35 °C (Hussain et al., 2011).

Table 5.1 Performance of the MFC at different syngas flow rates.

Test	$L(L_R)^{-1}d^{-1}$	Power output (mW/L_R)	R_{int} (Ω)	Effluent acetate (mg/L)	H_2 (%)	CH_4 (%)	CO (%)	CO tranformation (%)	H_2 tranformation (%)	Syngas tranformation (%)	CE (%)
1	8	33	49	123	25	10	23	88	86	87	20
2	10	16	94	76	32	12	45	73	63	73	12
3	6	34	45	98	26	8	18	92	88	91	26
4	4	11	173	24	14	17	21	97	98	98	9

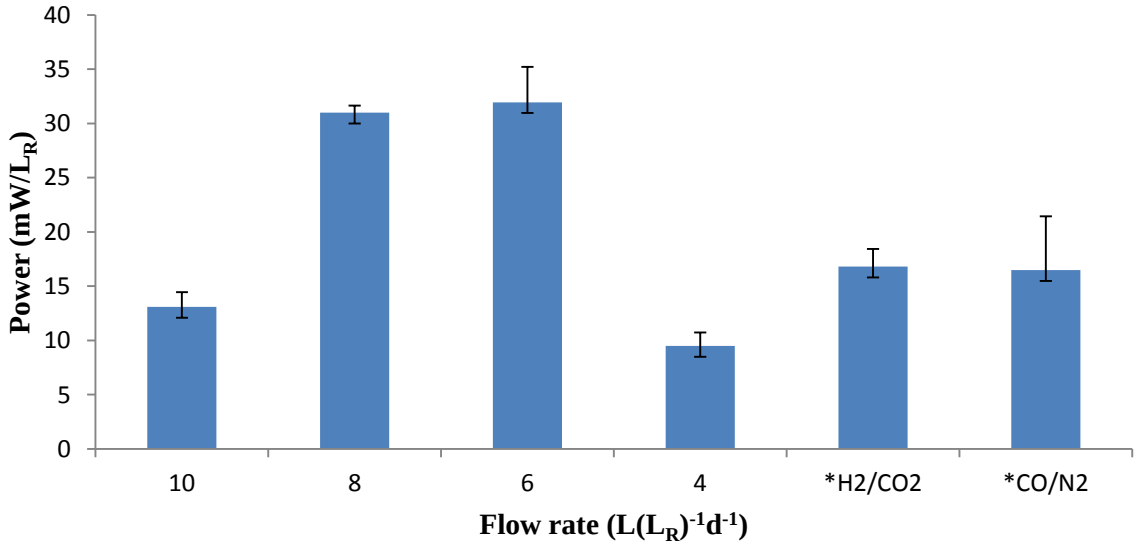

Figure 5.4 Average volumetric power output for MFC as different syngas flow rates, where H_2/CO_2 and CO/N_2 were fed at a flow rate of 6 L L_R^{-1} d^{-1}.

Decreasing the syngas flow rate to 6 L L_R^{-1} d^{-1}, restored the average power output to 32 ± 3.2 mW L_R^{-1} (Fig. 5.4). The syngas transformation efficiency increased to 91 % and CE rebounded to 26 % as compared to only 12 % CE observed at the highest syngas flow rate (Table 5.1). Also, R_{int} and P_{max} values obtained in the polarization tests were similar to those obtained at a flow rate of 8 L L_R^{-1} d^{-1} (45 Ω and 33 mW L_R^{-1} , respectively).

The syngas transformation efficiency was further improved to 98 % at a syngas flow rate of 4 L L_R^{-1} d^{-1} (Table 5.1). However, the average power output considerably decreased to 10 ± 1.3 mW L_R^{-1} (Fig. 5.4). Furthermore, CE also declined to 9%. As in the previous tests, the power output observed during MFC operation with the P/O algorithm agreed well with a P_{max} of 11 mW L_R^{-1} obtained in the polarization test (Fig. 5.3B). The polarization test also revealed an increase in internal resistance to 173 Ω.

Overall, the power output and the syngas/CO conversion efficiency were considerably higher than those reported for the mesophilic syngas-fed MFCs, which produced a maximum power output of 4.5-8.1 mW L_R^{-1} with a CO conversion efficiency of 50-55 % (Hussain et al., 2011; Mehta et al., 2010; Neburchilov et al., 2011). The maximum current density achieved under thermophilic conditions was in a range of 1.9–2.1 A m^{-2}, which was also above the maximum current density of 1-1.5 A m^{-2} observed for the mesophilic MFC in our previous studies (Hussain et al., 2011; Mehta et al., 2010). In fact, the performance of the thermophilic syngas-fed MFC was comparable to that reported for thermophilic MFCs fed with acetate and other organic substrates, which have been reported to have power outputs of 5-100 mW L_R^{-1} (Jong et al., 2006; Marshall & May, 2009; Wrighton

et al., 2008). Power density could be further increased by improvement in system design such as increase in anodic surface area (Di Lorenzo et al., 2010). For example, in our tests the anode occupied 25 mL of the 50 mL anodic chamber. By recalculating the volumetric power output per anode volume, a power density of 70-75 mW L^{-1}_{anode} was obtained.

Several reasons can be cited to explain improved MFC performance at thermophilic conditions. As described in the previous section, the supply of CO to the anodic liquid was improved. Also, the thermophilic conditions might affect the activation, ohmic, and diffusion losses at the anode. Indeed, the activation losses contribute to 5-10 % of the total internal resistance in a mesophilic MFC (Logan, 2008; Zhang & Liu, 2010). The electrochemical reaction rates might increase with increasing temperature thus leading to lower activation losses. Furthermore, temperature also affects the diffusion of the substrates in the anodic liquid thereby influencing the concentration losses, which account for 45-50 % of the total internal resistance of a MFC (Logan, 2008; Zhang & Liu, 2010). Likely, the operation of the syngas-fed MFC at thermophilic temperatures increased the transfer rate of CO and H_2 not only to the anodic liquid, but also facilitated the transport of the dissolved gasses through the stagnant liquid layer adjacent to the anode fibers, thus reducing the diffusion losses. Overall, the internal resistance of the thermophilic MFC at optimized performance was less than 50 Ω, whereas the mesophilic MFC had an internal resistance of above 120 Ω (Hussain et al., 2011; Mehta et al., 2010).

In addition to affecting the process electrochemistry, it can be hypothesized that the thermophilic conditions increased the activity of the carboxidotrophic microorganisms. Up to a certain temperature, the biomass growth and substrate conversion rates increase with

temperature according to the Arrhenius relationship. In general, the thermophilic microorganisms feature higher growth and reaction rates as compared to the mesophilic cultures (Min et al., 2008). Therefore, a higher carboxidotrophic activity could be expected at thermophilic temperatures. In a mesophilic CO-fed MFC the step of CO conversion to acetate appeared to limit the overall transformation rate (Mehta et al., 2010). Another advantage could be related to the reduced O_2 solubility at elevated temperatures. While most of the O_2 diffusing through the cathode surface is consumed by the cathodic reaction, the residual O_2 diffuses to the anodic liquid thus resulting in the inhibition of the electricigenic and carboxidotrophic populations (Oelgeschlager & Rother, 2008) as well as competing with the anode as the final electron acceptor (Liu & Logan, 2004). The presence of trace amounts of O_2 in the anodic chamber was observed to greatly impair the power output of mesophilic syngas-fed MFC (Hussain et al., 2011).

5.4.3 H_2 and CO tests

Analyses of the MFC effluent and off-gas composition at various syngas feeding rates led to the identification of several soluble and gaseous degradation products (Table 5.1). In particular, up to 123 mg L^{-1} of acetate was present in the effluent along with trace amounts (less than 5 mg L^{-1}) of propionate, methanol and ethanol. Notably, improved electricity production correlated with higher levels of acetate in the effluent. CH_4 was detected in all off-gas samples at concentrations varying between 10-17 %. The CH_4 concentration was inversely proportional to the power output (Table 5.1).

In order to evaluate the pathways leading to electricity generation, single electron donor (H_2 or CO) tests were performed. Prior to each test, the anodic compartment was fed solely

with N_2 for at least 12 h to flush out the metabolic products formed during the previous test. Feeding the MFC with H_2 produced an average power output of 17 ± 1.6 mW L_R^{-1} (Fig. 5.4), with CH_4 (16 %) and acetate (38 mg L^{-1}) observed to be the major degradation products (Table 5.1). The subsequent feeding of the MFC with CO produced a comparable power output of 20 ± 2.1 mW L_R^{-1} (Fig 5.4), with the presence of significant amounts of acetate (26 mg L^{-1}) in the effluent and H_2 (8 %), CH_4 (12 %) in the anode off-gas.

An additional test was performed to study CO conversion to volatile fatty acids and methane in the absence of the electricigenic activity. For this test the bioreactor used for K_La estimations was inoculated with the mixed thermophilic enrichment culture and then fed with CO at a flow rate of 4 L L_R^{-1} d^{-1}. The appearance of CH_4 in the off-gas and acetate in the effluent was observed after 5 days of operation. At a steady state, the off-gas contained 14 % CH_4, 13 % CO, and 6 % H_2, while the effluent mainly consisted of acetate (81 mg L^{-1}). The presence of H_2 in the off-gas of the CO-fed bioreactor was indicative of hydrogenogenic activity. The hydrogenogenic activity was also most likely to be present in the MFC, but its presence could not be confirmed since H_2 constituted 50 % of the syngas.

Overall, the single electron donor tests suggested the existence of at least two alternative pathways leading to acetate formation. One pathway apparently proceeded through acetate formation from CO by the acetogenic carboxidotrophic microorganisms, while in the second pathway acetate was formed from H_2 by the homoacetogenic microorganisms, as shown in Figure 5.5. In either case, acetate might serve as a carbon source for the electricigenic microorganisms.

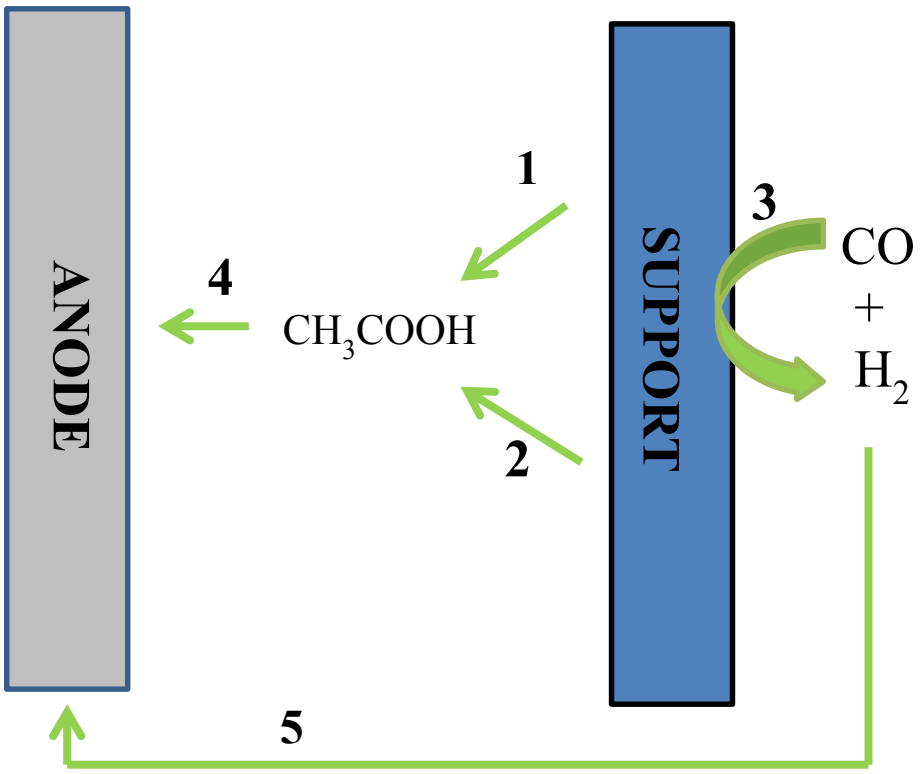

Figure 5.5 Pathways for generation of electricity in a thermophilic syngas-fed MFC as observed in this study. *1.* CO conversion to acetate by acetogenic carboxydotrophs, *2.* H_2 conversion to acetate by homoacetogens, *3.* CO conversion to H_2 by hydrogenogenic carboxydotrophs and *4 & 5.* Acetate and H_2 consumption by electricigenic microorganisms.

5.4.4 Microbial community analysis and pathways for electricity generation

To further elucidate the pathways of syngas transformation leading to electricity formation, the microbial communities of the anodic liquid, the anode, and the carbon felt support were subjected to DGGE of PCR amplified 16S rDNA fragments. Although some essential species may not be detected by this technique, it remains a widely used molecular technique for rapid assessment of the microbial communities present in complex samples, such as MFC anodes (Ercolini, 2004).

The identified eubacterial species were primarily present at the anode and were not found on the sample of the carbon felt used as a support for the silicone tubing. This felt was separated from the anode by a synthetic cloth and therefore was excluded from the electrical circuit. The anode population included sulfur-reducing bacteria belonging to the *Geobacteraceae* family (Table 5.2), such as *Geobacter sulfurreducens*, *Geobacter* sp.T32, and an uncultured *Geobacterium* originally isolated in a Microbial Electrolysis Cell (MEC) (Thygesen et al., 2011). This selective colonization of the anodic surface by the sulfur-reducing bacteria agreed with their ability to utilize the anode as the electron acceptor. The ability of the sulfur-reducing bacteria to grow or utilize CO as the sole source of energy has been reported (Sipma et al., 2006a). Other eubacterial populations identified at the anode largely consisted of uncultured bacteria which were originally isolated in MFCs, or MECs, or anaerobic digesters.

Homoacetogens belonging to the genus *Acetobacterium* were found to be present on the silicone tubing support (Table 5.2). The genus includes anaerobes capable of utilizing H_2 and CO_2 and forming acetate. *Acetobacterium wieringae* grows chemolithotrophically with H_2 and CO_2 forming acetic acid as the sole end product (Braun & Gottschalk, 1982). *Acetobacterium wieringae* have been widely identified in CO and syngas fed reactors (Sipma et al., 2006b; Van Houten et al., 2006) and were also found in the CO fed MFC described by Kim and Chang (2009).

Table 5.2 Eubacterial species identified in the syngas-fed MFC operated at 50 °C.

Identified Microorganism	Identity (%)	Origin / Growth Conditions	Hypothesized Activity in a syngas-fed MFC	Surface	Reference
Uncultured Bacterium	99	Industrial anaerobic digester	_	A,L	FJ462092*
Uncultured Bacterium	99	Microbial community of a biocathode in hydrogen producing MEC	_	A,L	(Croese et al., 2011)
Uncultured Bacterium	99	Isolated from the anaerobic tank of a water treatment plant	_	A,L	(Liu et al., 2010)
Uncultured Bacterium	99	Thermophilic anaerobic hydrid reactor degrading terephthalate	_	A,L	(Chen et al., 2004)
Uncultured Bacterium	99	Sulfate-reducing communities in a fluidized-bed reactor treating acidic metal and sulphate containing water	Electricigenesis	A	(Kaksonen et al., 2004)
Geobacter sp. T32	99	Anodic community of an MFC	Electricigenesis	A	(Michaelidou et al., 2011)
Geobacter sulfurreducen	99	Acetate	Electricigenesis	A	AB568596*
Uncultured *Geobacter*	99	MEC	Electricigenesis	A,L	(Thygesen et al., 2011)
Uncultured Bacterium	99	MFC	-	S,L	EF515355*
Acetobacterium wieringae	100	H_2/CO_2	Homoacetogenesis	S	(Braun & Gottschalk, 1982)
Uncultured *Acetobacterium*	98	Paddy soil	Homoactegenesis	S	FR774812*

Where: **S** denotes support, **L** denotes effluent liquid and **A** denotes anode
*GenBank accession no.

As mentioned above, acetate was observed to be the major metabolite throughout the MFC operation on syngas and also during the H_2 and CO tests. Moreover, the electricity generation was always accompanied by the presence of acetate in the anodic chamber (Table 5.1). Therefore, the identification of acetogens confirmed that at least one of the pathways for electricity production involved H_2 and CO conversion to acetate, and subsequent oxidation of acetate by electricigenic microorganisms (Fig 5.5). The ability of *G. sulfurreducens* to use acetate and H_2 for electricity generation in an MFC has previously been demonstrated (Bond & Lovley, 2003). Interestingly, the acetogenic species were detected on the carbon felt support, which was not connected to the electrical circuit, while all electricigenic species were found in the anode felt samples.

The presence of H_2 in the off-gas when the MFC was fed solely with a CO/N_2 mix, affirmed the presence of hydrogenogenic activity. It was also confirmed in the bioreactor test, when CO was fed and the presence of H_2 was detected in the off-gas. An increase in H_2 formation from CO at temperatures of 45-55°C has been reported (Sipma et al., 2006b). This H_2 derived from CO, along with the H_2 in the syngas mixture could be utilized directly by the electricigenic microorganisms and/or can be used for acetate formation by homoacetogens as described earlier.

The archaea identified in this study were methanogens (Table 5. 3). The ability to grow in the presence of CO or utilize CO as a sole source of energy has been reported in phylogenetically and physiologically diverse methanogenic groups (Oelgeschlager & Rother, 2008). The identified methanogens had the capacity for the sequential reduction of CO_2 to CH_4 in the hydrogenotrophic pathway, which might explain the significant amount of CH_4 in the anodic off-gas.

Table 5.3 Archaeal species identified in the syngas-fed MFC operated at 50 °C.

Identified Microorganism	Identity (%)	Origin / Growth Conditions	Hypothesized Activity in a Syngas-fed MFC	Surface	Reference
Methanothermobacter wolfeii	100	H_2/CO_2	Hydrogenotrophic Methanogenesis	S,L	(Winter et al., 1984)
Methanothermobacter thermautotrophicus	100	H_2/CO_2	Hydrogenotrophic Methanogenesis	S,L	(Nölling et al., 1993)
Uncultured Archaeon	100	Isolated from a thermophilic (53 °C) anaerobic biowaste fermenter	-	S,L	(Malin & Illmer, 2008)
Methanobrevibacter arboriphilicus	100	$H2/CO_2$	Hydrogenotrophic Methanogenesis	A,S,L	(Watanabe et al., 2004)
Uncultured Archaeon	100	Isolated from a Microbial digester	-	A,S,L	(Wagner et al., 2011)
Uncultured *Methanobrevibacterium*	99	Isolated from a high temperature natural gas field in Japan	Hydrogenotrophic Methanogeneis	S,L	(Mochimaru et al., 2007)
Uncultured *Methanobacterium*	99	Isolated under low-hydrogen conditions	Hydrogenotrophic Methanogenesis	A,S,L	(Sakai et al., 2009)
Uncultured *Methanobacterium*	99	Isolated from a high temperature petroleum reservoir	Hydrogenotrophic Methanogenesis	A,S,L	(Kobayashi et al., 2011)

Where: **S** denotes support, **L** denotes effluent liquid and **A** denotes the anode

Methanothermobacter wolfeii, *Methanothermobacter thermautotrophicus* and *Methanobrevibacter arboriphilicus* were identified. These species utilize H_2 and CO_2 for growth and CH_4 formation (Daniels et al., 1977;Wasserfallen et al., 2000; Winter et al., 1984). In addition, the ability of *M. thermoautotrophicus* and *M. arboriphilicus*, to remove CO in the gas phase while growing on CO_2 and H_2 has been reported (Daniels et al., 1977). *M. thermoautotrophicus* could utilize CO as the sole energy source by disproportionating CO to CO_2 and CH_4. This ability could be attributed to the presence of carbon monoxide dehydrogenase (CODH) and acetyl-CoA synthase (ACS) in the microorganisms, the two metalloenzymes fundamental for growth on CO (Oelgeschlager & Rother, 2008). The other uncultured archaea identified in the MFC belonged to the genera *Methanobacterium* and *Methanobrevibacterium*, that again possess the hydrogenotrophic pathway for CH_4 formation (Wasserfallen et al., 2000). Overall, the methanogens must have competed with the electricigenic microorganisms for the electron donor, resulting in a low CE. A similar observation was also made in our previous studies (Hussain et al., 2011; Mehta et al., 2010) and in the study of Kim et al. (2005), where a 30 % increase in CE was observed, when a methanogenic inhibitor was utilized. MFC operation at a retention time of 5 days (liquid inflow of 10 mL d^{-1}) might have contributed to the proliferation of methanogens in the anodic liquid. This implies that the CE of the system could be further improved by the optimization of the influent flow rate to find a right balance between the retention of intermediates utilized for electricity production and the wash out of the planktonic methanogenic microorganisms.

We propose that the methanogenesis, which does not result in electricity generation, could be eliminated by the development of thermophilic syngas-fed MFC with an

engineered microbial consortium. A thermophilic syngas-fed MFC seeded with pure cultures of electricigenic microorganism such as *Thermincola ferriacetica*, a CO-tolerant, acetate utilizing bacterium, in a syntrophic relationship with homoacetogens and carboxydotrophic microorganisms might be capable of achieving higher power densities due to the elimination of methanogenesis and additionally due to the higher catalytic activity of the thermophilic microorganisms. Thermophilic acetate fed MFC seeded with a pure culture of *Thermincola ferriacetica* was recently demonstrated to achieve a CE of ~ 97 %. The power densities reported were comparable or even higher than the thermophilic MFCs inoculated with anaerobic sludge (Marshall & May, 2009).

5.5 Conclusion

This study for the first time demonstrates electricity generation in a thermophilic MFC fed with syngas. The thermophilic conditions led to a higher power density and improved syngas transformation efficiency as compared to a similar MFC operated under mesophilic conditions. The Coulombic efficiency was also improved to 20-26% as compared to 6-9% reported for the mesophilic MFC. Significant methane production remains the main obstacle to a higher Coulombic efficiency.

The analyses of the effluent and off-gas compositions in combination with DGGE analysis of 16S rDNA fragments indicated the existence of several concurrent pathways leading to electricity formation. Electricity generation predominantly takes place in a two-step process in which syngas is converted to acetate and then acetate is oxidized by the electricigenic microorganisms to produce electricity. This pathway is accomplished by a

syntrophic association between electricigenic, carboxydotrophic and homo-acetogenic microorganisms.

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Connecting text

Chapter 5 demonstrated electricity production from syngas under thermophilic conditions for the first time. Themophilic conditions considerably enhanced the MFC power output and Coulombic efficiency (CE). However, practical applications of CO/syngas-fed MFCs would require much higher volumetric efficiencies. We hypothesized that volumetric efficiency of a CO/syngas-fed MFC could be increased by further improvements in the MFC design. Accordingly, the following study (Chapter 6) evaluated ability of a multi-electrode design containing three anodes and two cathodes to achieve improved volumetric efficiency on syngas at several operating temperatures and anode-cathode arrangements.

This study is to be published in *Journal of Chemical Technology and Biotechnology* (2013).

CHAPTER 6

Electricity production from synthesis gas in a multi-electrode microbial fuel cell

6.1 Abstract

This study demonstrated electricity production in a multi-anode/cathode MFC fed with synthesis gas (syngas) as the sole electron donor at several operating temperatures ranging from 37 °C to 50 °C. In addition to providing a compact system design, the adoption of a multi-electrode configuration considerably enhanced the system performance. A maximum power density of 33 mW L_R^{-1} (normalized to the anodic compartment volume) and a Coulombic efficiency (CE) of 43 % was achieved at an operating temperature of 37 °C. The MFC performance at elevated temperatures was restricted by low thermophilic microbial activity. Consequently, a much lower power density of 10 mW L_R^{-1} and a CE of 15 % were obtained at 50 °C. The MFC power density was also greatly impacted by the anode-cathode arrangement. Among the several electrode arrangements tested in this study, the highest power density was achieved in a three anode-two cathode (3A-2C) arrangement.

Keywords: Microbial fuel cell; Synthesis gas; Carbon monoxide; Multi-electrode design

6.2 Introduction

The utilization of syngas as an electron donor for electricity production in a microbial fuel cell (MFC) represents a promising alternative technology for renewable energy production that could offer several advantages including a high transformation efficiency, broad range of operating conditions, a high bio-catalytic activity and considerable resistance to poisoning by fuel impurities (Hussain et al., 2011a). Electricity production in an MFC directly fed with syngas has been recently demonstrated (Hussain et al., 2011b; Mehta et al., 2010). Improvements in the design of syngas-fed MFCs have led to a substantial increase in their volumetric power output, syngas transformation and Coulombic efficiency (CE) (Hussain et al., 2011b; Neburchilov et al., 2011). However, practical applications of such systems would require much higher volumetric efficiencies.

Electricity production from syngas has so far been tested in a single-anode/cathode MFC design (Hussain et al., 2011a). An efficient way of increasing power production in an MFC might be with the adoption of a multi-electrode design. Multi-anode/cathode MFCs fed with acetate, waste water and other liquid organic substrates have been reported to attain improved volumetric efficiencies (Jiang et al., 2011; Jiang et al., 2010). However, electricity production from syngas is a culmination of complex electrochemical, transport phenomena and microbiological interactions, all of which are greatly affected by system architecture and design, such that the overall outcome of enhanced system performance is uncertain and thus warrants a systematic experimental study to examine the applicability of a multi-anode/cathode design to achieve high volumetric efficiency on syngas.

This study presents the first attempt to operate an MFC with a multi-electrode design (meMFC) on syngas. First, the impact of the proposed system design including different anode-cathode arrangements on power output was evaluated in an acetate-fed meMFC. Subsequently, a similar meMFC was operated on syngas at temperatures of 37 °C, 45 °C and 50 °C, and the impact of different electrode arrangements on power output was simultaneously examined.

6.3 Material and Methods

6.3.1 Medium composition and analytical methods

The composition of the stock solution of nutrients used in this study was (in g L^{-1}): 1.87 NH_4Cl, 14.81 KCl, 6.40 K_2HPO_4, 4.07 KH_2PO_4 and 0.215 yeast extract. The trace metal stock solution was prepared according to Tartakovsky et al.(2008) and contained (in g/L): 2 $FeCl_2$4H_2O, 0.05 H_3BO_3, 50 $ZnCl_2$, 0.03 $CuCl_2$, 0.5 $MnCl_2$4H2O, 0.5 (NH_4)6Mo7$O_2$44H2O, 0.5 $AlCl_3$, 0.5 $CoCl_2$6H_2O, 0.5 $NiCl_2$, 0.5 EDTA, and concentrated HCl (1 mL). The chemicals used were of analytical grade. The stock solutions were filter-sterilized with a 0.22 µm filter (Fisher Scientific, Ottawa, ON, Canada) and maintained at 4°C until use. The influent solution was prepared by adding 35 mL of the nutrient solution and 1 mL of the trace metal solution to 1 L of de-ionized water. This solution had a conductivity of 12 -14 mS cm^{-1}. For meMFC operation on acetate, an acetate solution was prepared by adding 40 g of sodium acetate to 1 L of de-ionized water.

Acetate and other volatile fatty acids (VFAs) in the effluent stream were analyzed using an Agilent 6890 gas chromatograph (Agilent Technologies Inc, Santa Clara, CA, USA) equipped with a flame ionization detector. The off-gas flow rate was measured using the

MilliGascounter^{TM} (Ritter Apparatus, Bochum, Germany). The off-gas composition was measured using an HP 6890 gas chromatograph (Hewlett Packard, Palo Alto, CA) equipped with a thermal conductivity detector and a 5 m x 2.1 mm Carboxen-1000 column (Supelco, Bellafonte, PA) with argon as the carrier gas. Further details of the analytical methods are provided in Guiot et al. (2011).

6.3.2 MFC design, operation, and characterization

Two continuous flow multi-anode/cathode MFCs to be fed with acetate and syngas respectively, were constructed from custom made nylon plates. The single chamber acetate-fed meMFC (hereby referred to as meMFC#1) consisted of a 50 mL anodic compartment that contained two carbon felt anodes (designated as A1, A2) each measuring 10 cm x 5 cm x 0.5 cm (SGL Canada, Kitchener, ON, Canada). Titanium rods with an Ir-MMO coating (Magneto Special Anodes, B.V, Netherlands) inserted into the felt were used as current collectors. Commercially available gas diffusion cathodes containing MnO_2 as a catalyst (Electric Fuel Ltd., Bet Shemesh, Israel) were used in this study. Two cathodes (designated as C1, C2), each measuring 10 cm X 5 cm were placed next to the anodes, thus representing a two anode-two cathode configuration (2A-2C). The cathodes were separated from the anodes by a non-conductive synthetic cloth. The meMFC#1 was equipped with lines for influent, effluent, liquid recirculation and gas exit, as shown in Fig. 6.1. It was inoculated with 10 mL of a homogenized mesophilic anaerobic sludge treating agricultural wastes (Lassonde Inc., Rougement, QC, Canada). Acetate solution was fed at a rate of 5 mL d^{-1} with a syringe pump. The solution of nutrients and microelements was continuously fed at a rate of 75 mL d^{-1} utilizing a peristaltic pump.

The 100 mL, single chamber syngas-fed meMFC (hereby referred to as meMFC#2) was similar in design to the acetate-fed meMFC. For syngas delivery in the anodic liquid, silicone tubing coiled around a third layer of carbon felt anode (designated as A3) was placed in the middle of the two pre-installed anodes (Fig. 6.1). The silicone tubing (VWR International LLC, Radnor, PA, USA) had a thickness of 90 µm, an external diameter of 1.76 mm and a total surface area of 75 cm^2. As shown in Fig. 6.1, the tubing was operated under a dead-end mode, with its inlet connected to the gas line, while the outlet was connected to a manometer. The solution of nutrients and microelements was fed at a rate of 20 mL d^{-1}. To evaluate the effect of temperature on the system performance, meMFC#2 was operated at temperatures of 37 °C, 45 °C and 50 °C. The anodic liquid was maintained at the desired temperature by heating the liquid in the external recirculation loop of the MFC. To simulate syngas feeding, CO & H_2 (50:50 v/v) were simultaneously fed at the desired flow rate. At 45 °C and 50 °C, meMFC #2 was fed with a syngas flow rate of 2 L L_R^{-1} d^{-1} (expressed as L of syngas fed per L of anodic chamber (L_R^{-1}) volume per day). Two additional flow rates of 1.2 and 3 L L_R^{-1} d^{-1} were tested at 37 °C. Each flow rate was tested for a period of at least 5 days.

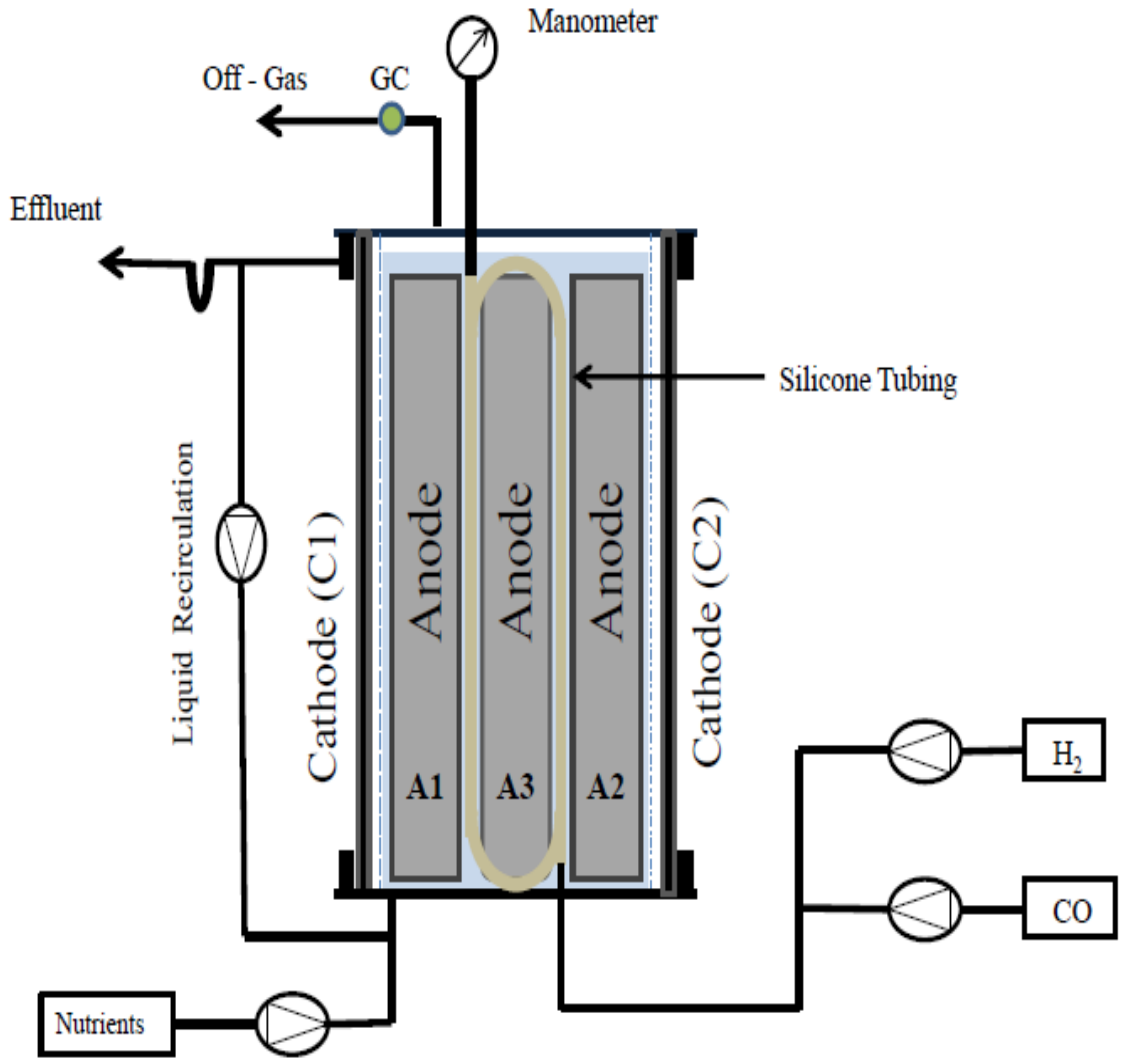

Figure 6.1 Diagram of the experimental set-up of syngas-fed multi-anode/cathode MFC (meMFC#2), where GC represents the gas counter. The meMFC#1 setup lacked H_2 and CO lines and middle anode (A3) with silicone tubing.

During start-up, meMFC#1 was operated in the two anode-two cathode (2A-2C) arrangement, in which all anodes were interconnected by a wire and two cathodes were also electrically connected by a wire. Similarly, meMFC#2 was initially operated under three anode-two cathode (3A-2C) arrangement (Fig. 6.2A). Both MFCs were started at a constant

external resistance of 500 Ω. Once a steady power output was observed, the external resistance was optimized using the Perturbation and Observation MPPT algorithm, which constantly adjusted the external resistance to maximize the MFC power output in real time (Woodward et al., 2009). The MFC voltage was measured and recorded at 20 min intervals using Labjack U12 (Labjack Corp., Lakewood, CO, USA). Different anode-cathode arrangements were then tested by varying the external electrical connections and maintaining each arrangement for a period of at least 6 h to stabilize output voltage and current. All tested electrode arrangements for meMFC#1 and meMFC#2 are listed in Table 6.1. The 3A-2C and 1A-1C electrode arrangements for meMFC#2 are illustrated in Fig. 6.2A and 6.2B, respectively. Polarization tests were carried out at the end of each test following the procedure described in Logan et al. (2008).

Coulombic efficiency (CE) of the syngas-fed meMFC was calculated as:

$$CE = \frac{I\Delta t}{Fn\left(\frac{M_{CO,in} - M_{CO,out}}{M_{CO}} + \frac{M_{H2,in} - M_{H2,out}}{M_{H2}}\right)} 100\% \quad \text{..... (6.1)}$$

Where, I is the average current (A), Δt is the time interval during which the current was measured (s), M_{CO} is the molar mass of CO (g/mol), M_{H2} is the molar mass of H_2 (g/mol), F is the Faraday constant (C/mol-e^-), n is the number of electrons exchanged (mol-e^-/mol), $M_{CO,in}$ and $M_{H2,in}$ are the amounts of CO and H_2, respectively, supplied (g) to meMFC#2 during Δt, $M_{CO,out}$ and $M_{H2,out}$ are the amounts of CO and H_2, respectively, recovered (g) in the off-gas during Δt.

Table 6.1 List of different electrode arrangements tested for acetate and syngas-fed multi-anode/cathode MFCs (meMFC#1 and meMFC#2).

Test	**Substrate**	**Flow rate** ($L\ L_R^{-1}\ d^{-1}$)	**Electrode Arrangement**	**Description**	**Temperature** (°C)
1	Acetate	-	2A-2C	(A1,A2)-(C1,C2) electrically connected	22
2	Acetate	-	2A-1C (L)	(A1,A2)-(C1) electrically connected	22
3	Acetate	-	2A- 1C (R)	(A1,A2)-(C2) electrically connected	22
4	Acetate	-	1A-1C (L)	(A1)-(C1) electrically connected and optimized separately using P/O algorithm	22
5	Acetate	-	1A-1C (R)	(A2)-(C2) electrically connected and optimized separately using P/O algorithm	22
6	Syngas	1.2, 2, 3	3A-2C	(A1, A2, A3)-(C1,C2) electrically connected	37, 45, 50
7	Syngas	1.2, 2, 3	3A-1C (L)	(A1, A2, A3)-(C1) electrically connected	37,45, 50
8	Syngas	1.2, 2, 3	1A-1C (L)	(A1)-(C1) electrically connected	37, 45, 50
9	Syngas	1.2, 2, 3	3A-1C (R)	(A1, A2, A3)-(C2) electrically connected	37, 45, 50
10	Syngas	1.2, 2, 3	1A-1C (R)	(A2)-(C2) electrically connected	37, 45, 50

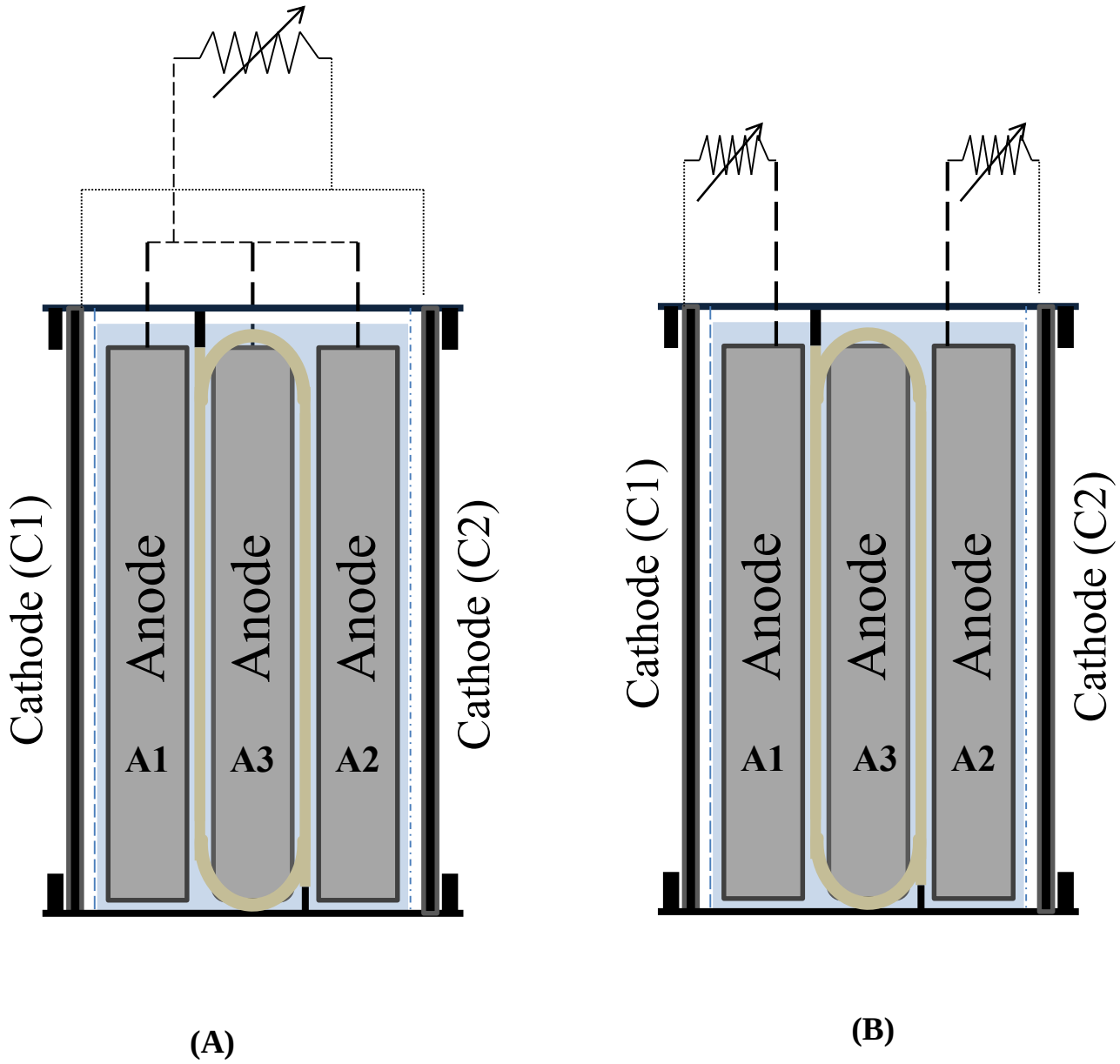

Figure 6.2 Schematic representation of (A) 3A-2C and (B) 1A-1C (one anode–one cathode, middle anode is not connected) electrode arrangement for syngas-fed multi-anode/cathode MFC (meMFC#2).

6.3.3 Protein quantification

Protein quantification was performed at the end of meMFC#2 operation to evaluate the cell density in the anodic biofilm. Pieces of carbon felt measuring 1 cm x 1 cm x 5 cm were cut from the middle and bottom of each anode using a surgical scissor and stored at -20 °C until use. Protein analysis was performed following the procedure described in Gil-Carrera et al. (2011) and expressed as mg of protein/mL of anode (L_A^{-1}).

6.4 Results and Discussion

6.4.1 Impact of electrode arrangements on power output of meMFC#1

To validate the ability of our MFC design to achieve enhanced power outputs, the proposed multi-electrode design (meMFC) was first tested on acetate. The test was initiated with meMFC #1 being operated in a 2A-2C arrangement at an external load of 500 Ω. Within 2 days of initiation of the test, the output voltage increased from 0 to 100 mV, corresponding to a power output of 0.4 mW L_R^{-1} . From day 3 onwards, the on-line perturbation/observation (P/O) algorithm was utilized to optimize the external resistance, thus maximizing the power output at all times (Woodward et al., 2009). A steady power output of 73 ± 5 mW L_R^{-1} was obtained after 5 days of operation. To estimate the internal resistance (R_{int}), the open circuit voltage (OCV), and the maximum power output (P_{max}), polarization tests were performed on day 7. An OCV value of 620 mV was measured and a R_{int} of 21 Ω was estimated based on the polarization curve shown in Fig. 6.3A. Accordingly, P_{max} of 82 mW L_R^{-1} was obtained, as shown in Fig. 6.3B. The R_{int} estimation agreed well with the optimal value of R_{ext} chosen by the P/O algorithm, which varied between 15-25 Ω.

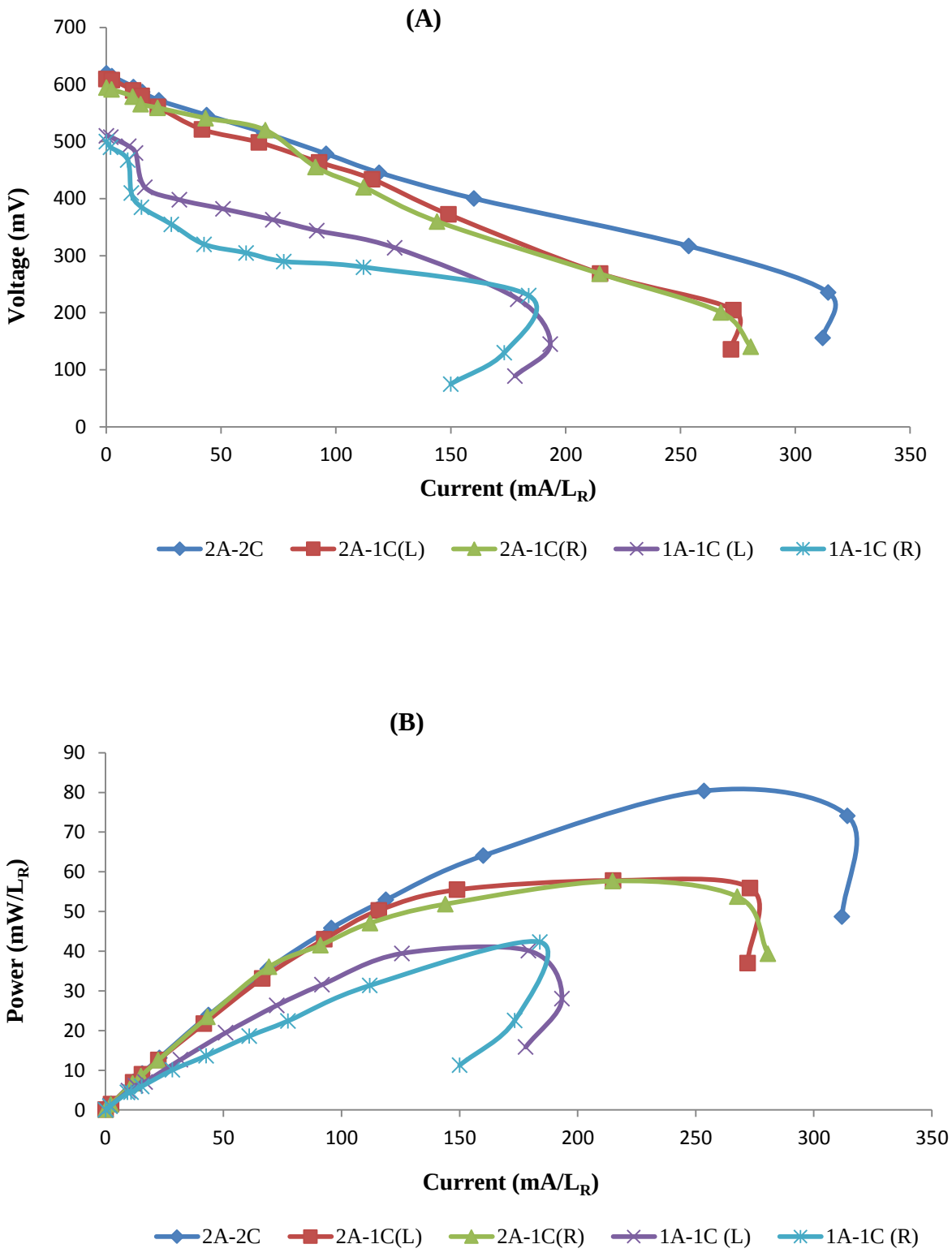

Figure 6.3 (A) Polarization and (B) power curves for acetate-fed multi-anode/cathode MFC (meMFC#1).

The impact of different anode-cathode arrangements on power output of meMFC#1 were studied by comparing different external circuit connections. Five different circuit connections as outlined in Table 6.1 were tested. Highest power outputs and lowest R_{int} values were obtained in 2A-2C configuration (two interconnected anodes and two interconnected cathodes). A reduction in number of interconnected electrodes negatively impacted meMFC#1 performance. Operating meMFC#1 in a two anode-one cathode arrangement (2A-1C) resulted in up to 30 % reduction in power density. A reduced maximum power output of 58 mW L_R^{-1} was obtained in 2A-1C(L) arrangement (Fig. 6.3B, L denotes left-side cathode), compared to 82 mW L_R^{-1} achieved in 2A-2C configuration. Similarly, the power density reduced to 57 mW L_R^{-1} when operated in 2A-1C(R) electrode arrangement (Fig. 6.3B, R denotes right-side cathode). The decrease in power output was manifested by 25-30 % increase in R_{int} to 30-32 Ω, compared to 24 Ω in 2A-2C configuration.

A reduction in the number of interconnected anodes had an equally significant effect on power density. The operation of meMFC#1 in a 1A-1C(L) arrangement resulted in a maximum power density of 39 mW L_R^{-1}, a reduction of ~ 33 % compared to the power density obtained in 2A-1C(L) arrangement (Fig. 6.3B). Similarly, the power density of 40 mW L_R^{-1} obtained in 1A-1C(R) arrangement was 30 % less than that observed in 2A-1C(R) arrangement (Fig.6.3B). Notably, the power produced by each anode-cathode pair was almost equal to the power produced by single-anode/cathode MFCs fed with acetate (Pinto et al., 2011). Also, the cumulative sum of the power densities for each electrode pair

(39 mW L_R^{-1} + 40 mW L_R^{-1}) was almost equal to the power density achieved in 2A-2C arrangement (82 mW L_R^{-1}), thereby further substantiating the ability of multi-electrode MFCs to achieve high power outputs with lower reactor volumes.

Overall, the power output of meMFC#1 was almost two times higher in comparison to single-anode/cathode MFCs (20-45 mW L_R^{-1}) operated on acetate in our previous tests (Pinto et al., 2011; Pinto et al., 2010). The power output was also higher in comparison to multi-anode/cathode MFCs used in other studies, which have been reported to have power outputs of 40-60 mW L_R^{-1} at similar organic loads (Ahn andLogan, 2012; Jiang et al., 2011; Jiang et al., 2010). These results clearly elucidated the ability of our meMFC design to achieve an improved performance, which is most likely due to improved system design leading to a reduced internal resistance.

6.4.2 Impact of temperature and electrode arrangements on meMFC#2 operation on syngas

Following meMFC#1 tests on acetate, a second meMFC (meMFC#2) was operated on syngas. The syngas tests were aimed at evaluating the impact of temperature and electrode arrangements on the performance of syngas-fed MFCs. Consequently, meMFC#2 was operated at several operating temperatures, and the effect of different electrode arrangements was examined. The meMFC#2 operation was initiated with the system being operated at a temperature of 50 °C and fed with syngas at a flow rate of 2 L L_R^{-1} d^{-1}. As found to be optimal in previous test, during start-up of meMFC#2 all anodes and cathodes were electrically connected (3A-2C arrangement) as shown in Fig. 6.2A. No electricity production was

observed prior to inoculation (abiotic conditions). After the inoculation, a long start-up period was required before any significant voltage output was observed. A voltage output of 100 mV, corresponding to a power output of 0.2 mW L_R^{-1} was obtained 16 days after inoculation. From this point on, the P/O algorithm was utilized to optimize the external resistance at all times. An additional 10 days were required before the power output stabilized to 6.4 ± 1.8 mW L_R^{-1} (Fig. 6.4). Polarization tests performed after 30 days of meMFC#2 operation at 50 °C in 3A-2C electrode arrangement produced a maximum power output (P_{max}) of 10 mW L_R^{-1} at R_{int} of 84 Ω (Fig. 6.5). A Coulombic efficiency (CE) of 15 % and syngas transformation efficiency of 53 % (Table 2) were obtained. This performance of meMFC #2 was considerably lower to that observed in our previous tests using a thermophilic syngas-fed MFC with a single-anode/cathode design, where power densities of up to 30-35 mW L_R^{-1} and syngas transformation efficiency of 90-95 % was observed (Hussain et al., 2012). Electricity production from syngas in an MFC proceeds through multi-step concurrent pathways, which are accomplished by a syntrophic association among electricigenic, carboxydotrophic and homo-acetogenic microorganisms. The conversion of CO and H_2 to acetate, followed by its oxidation by CO tolerant-electricigenic microorganisms represents the most prominent pathway for electricity production (Hussain et al., 2012). The low CO and syngas transformation efficiency and consequently, the low effluent acetate concentration (Table 6.2) suggested that electricity production in meMFC#2 was either restricted by insufficient syngas transfer to the anodic liquid leading to substrate limitations, or by the low thermophilic potential of the mesophilic inoculum used in this study.

Table 6.2 Performance of syngas-fed multi-anode/cathode MFC (meMFC#2) at different operating temperatures and syngas flow rates.

Temperature (°C)	Flow rate ($LL_R^{-1}d^{-1}$)	Power Output (mW L_R^{-1})	R_{int} (Ω)	Effluent Acetate (mg L^{-1})	CO (%)	CH_4 (%)	H_2 (%)	CO transformation (%)	H_2 transformation (%)	Syngas transformation (%)	CE (%)
50	2	10	84	19	40	7	37	53	57	53	15
45	2	15	45	31	27	13	20	69	85	70	23
37	2	33	24	61	9	15	14	90	96	90	43
37	1.2	17	35	26	5	13	14	91	97	93	26
37	3	12	65	44	12	10	9	77	86	77	21

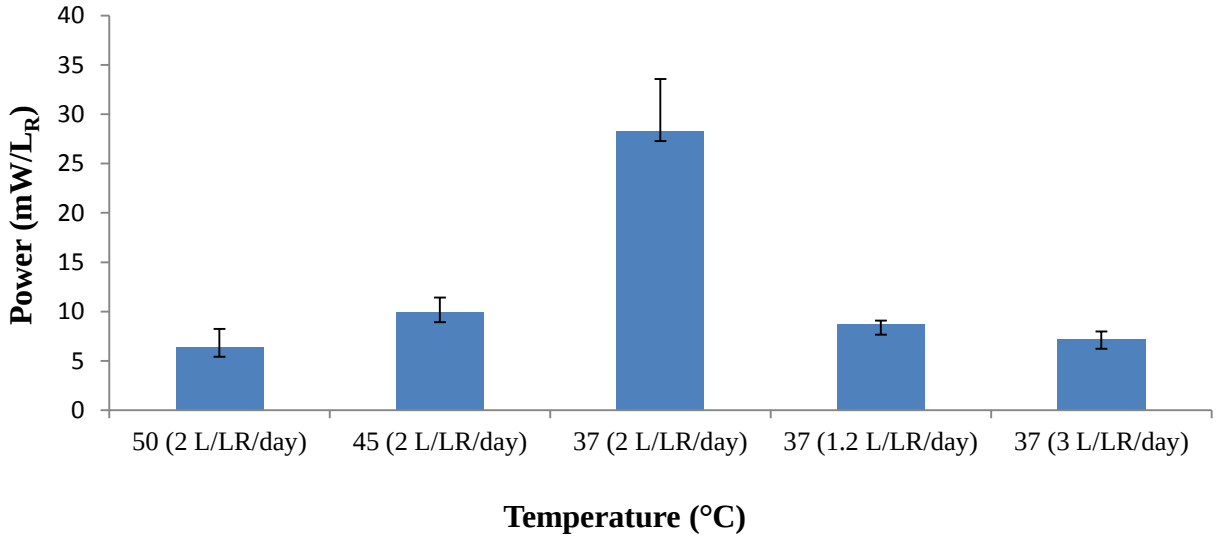

Figure 6.4 Average volumetric power output at different operating temperatures and syngas flow rates for syngas-fed multi-anode/cathode MFC (meMFC#2).

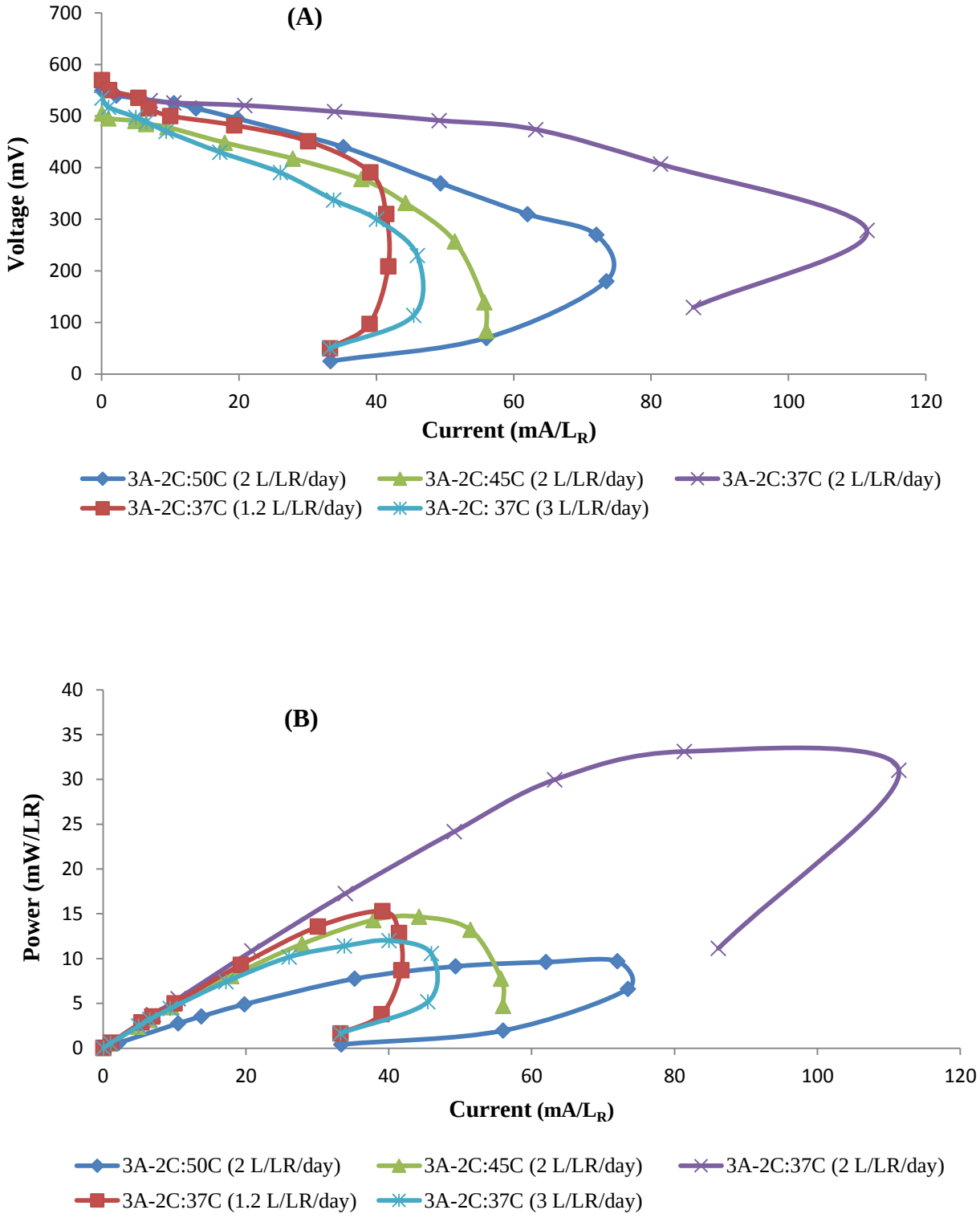

Figure 6.5 (A) Polarization and (B) power curves at different operating temperatures and syngas flow rates for syngas-fed multi-anode/cathode MFC (meMFC#2) in a 3A-2C electrode arrangement.

Gas-liquid mass transfer limits the conversion rates in bioprocesses that utilize sparingly soluble gases such as syngas. MFC operation on syngas at thermophilic temperatures is expected to further limit syngas transfer in the anodic liquid; however, this is counteracted by increase in the mass transfer rate (Guiot et al., 2011; Hussain et al., 2011a). To examine if the electricity production in meMFC#2 was limited by substrate availability, dissolved gas concentrations in the anodic liquid were measured. While the dissolved CO concentrations could be measured, the measurement of dissolved H_2 was unsuccessful due to its low solubility in the liquid and the insufficient detection limit of the analytical method. A dissolved CO concentration of ~ 6 mg/L was obtained, which was similar to that measured in mesophilic syngas-fed MFCs fed with similar gas flow rate (Hussain et al., 2011b). Though the dissolved H_2 concentration could not be measured, we hypothesize that the dissolved H_2 concentration should have been similar to that under mesophilic conditions. Also, the ability of silicone tubing systems to efficiently transfer H_2 to the anodic liquid under thermophilic conditions has been previously demonstrated (Hussain et al., 2012; Hussain et al., 2011b). Based on these observations, and the near-saturation concentration of dissolved CO in the anodic liquid of meMFC#2, it was concluded that sufficient amounts of substrate were available for microbial metabolism and the electricity production in meMFC#2 was not restricted by the MFC design. The power output of meMFC#2 at 50°C was conceivably restricted by the low carboxydotrophic and homo-acetogenic activity under thermophilic conditions. Additionally, to evaluate the electricigenic potential of the anodic bacterial communities at thermophilic temperatures, 1 mL of acetate solution was injected in the anodic compartment of meMFC#2. Contrary to previous observations, where acetate injection always led to an instant increase in power output of syngas-fed MFCs (Hussain et

al., 2011b; Mehta et al., 2010), acetate addition in this case had no significant effect on the power output of meMFC#2. This indicated the low electricigenic activity at thermophilic temperatures, which might have further restricted electricity production at 50 °C. Also, repetitive inoculations had no significant effect on meMFC#2 power output, clearly elucidating the low thermophilic potential of the mesophilic inoculum used in this study.

Lowering the operating temperature to 45 °C augmented the average power output to 10 ± 1.5 mW L_R^{-1} (Fig. 6.4), with a corresponding increase in CE to 23 %. Polarization experiments performed after 4 days of power stabilization, revealed a P_{max} of 15 mW L_R^{-1} (Fig. 6.5B) at a reduced R_{int} of 45 Ω compared to 84 Ω at 50 °C (Table 6.2). The improved performance of meMFC#2 could be attributed to the increased microbial activity at the mesophilic operating temperature of 45 °C. This is corroborated by the increase in syngas transformation efficiency to 70 % and the increased concentration of acetate in the MFC effluent to 31 mg L^{-1} (Table 6.2). An injection of 1 mL of acetate solution in the anodic chamber further raised the power output to 18 mW L_R^{-1} , indicating an increased electricigenic activity at mesophilic temperatures.

Considering that the mesophilic microorganisms have an optimal growth and activity between 35-40 °C, the performance of meMFC#2 was also evaluated at 37 °C. The temperature reduction from 45 °C to 37 °C considerably enhanced the system performance. A steady power output of 28 ± mW L_R^{-1} (Fig. 6.4) was obtained within 3 days of operation at 37 °C. Polarization curves acquired towards the end of meMFC#2 operation at 37 °C, affirmed the enhanced performance with a maximum power density of 33 mW L_R^{-1} (Fig.

6.5B), the highest observed for a syngas-fed MFC under mesophilic conditions. The enhanced power density was manifested by a substantial reduction in R_{int} to 24 Ω and an increased syngas transformation and Coulombic efficiency to 90 % and 43 %, respectively (Table 6.2).

To further maximize syngas consumption and meMFC#2 performance, additional syngas flow rates were tested at 37 °C. First, the syngas flow rate was reduced to 1.2 $L\,L_R^{-1}\,d^{-1}$. This reduction in flow rate marginally increased the syngas transformation efficiency to 93 %, but decreased the average and maximum power outputs to 9.2 ± 0.4 mW L_R^{-1} and 17 $mW\,L_R^{-1}$ (Fig. 6.4, 6.5), respectively. The increased R_{int} of 35 Ω and low acetate concentration in the effluent suggested that electricity generation was plausibly restricted by low carbon source concentrations. As previously mentioned, acetate serves as the primary intermediate for electricity production in a syngas-fed MFC, with low acetate levels in the anodic liquid at lower syngas flow rates limiting electricity generation (Hussain et al., 2012; Hussain et al., 2011b). Also, an increase in R_{int} under substrate limited conditions has been previously reported (Ieropoulos et al., 2010; Zhang andLiu, 2010).

Following the 1.2 $L\,L_R^{-1}\,d^{-1}$ test, the syngas flow rate was stepped-up to 3 $L\,L_R^{-1}\,d^{-1}$. This increase in flow rate significantly impaired meMFC#2 performance. The average power output was reduced to 7.2 ± 1 $mW\,L_R^{-1}$ (Fig. 6.4) and the syngas transformation efficiency dropped to 77 %. The R_{int} increased to 65 Ω, with a corresponding decrease in the maximum power output to 12 $mW\,L_R^{-1}$, which was deduced from the polarization curves obtained at the end of 3 $L\,L_R^{-1}\,d^{-1}$ test (Fig. 6.5). This reduced performance could be attributed to the

increased levels of dissolved CO in the anodic liquid at high syngas flow rates, which might have inhibited the carboxydotrophic and electricigenic microorganisms, thereby impeding MFC performance (Hussain et al., 2011b; Mehta et al., 2010). Consequently, flow rates above $3\ L\ L_R^{-1}\ d^{-1}$ were not tested.

Overall, meMFC#2 operation at different temperatures demonstrated syngas consumption and syngas dependant bioelectrochemical activity at a wide temperature range of 37 to 50 °C. The power output and syngas/CO conversion efficiency obtained in meMFC#2 during mesophilic operation was considerably higher to that observed for mesophilic syngas-fed MFCs with a single-anode/cathode design, which produced maximum power outputs of 4.8-8.1 $mW\ L_R^{-1}$, with a CO conversion efficiency of 50-55 % (Hussain et al., 2011b; Mehta et al., 2010). The maximum current density for meMFC#2 at 37 °C was in the range of 1.0 – 1.2 Am^{-2} (0.1-0.12 $A\ L_R^{-1}$) which was also well above the current density of 0.4 – 0.5 Am^{-2} (0.04 – 0.05 $A\ L_R^{-1}$) observed for mesophilic syngas-fed MFCs in our previous studies (Hussain et al., 2011b; Mehta et al., 2010; Neburchilov et al., 2011). In fact, the volumetric densities achieved in meMFC#2 at 37 °C was comparable to the mesophilic multi-anode/cathode MFCs fed with acetate, waste water and other organic substrates that have been reported to have power outputs in the range of 15-45 $mW\ L_R^{-1}$ with similar MFC dimensions and design (Ahn andLogan, 2012; Fan et al., 2007; Jiang et al., 2010). In addition to the increased volumetric densities, the utilization of a multi-anode/cathode configuration led to a significant enhancement in Coulombic efficiency on syngas. The maximum CE of 40-43 % obtained in this study was considerably higher than

those previously reported for mesophilic (CE of 6-8 %) and thermophilic (CE of 20-26%) syngas-fed MFCs (Hussain et al., 2012; Hussain et al., 2011b). These results clearly indicate that power generation and CE on syngas can be efficiently enhanced by the adoption of a multi-anode/cathode configuration and provides a promise of further improving power generation by integrating multiple electrodes. Practical applications would require the stacking of multiple large-scale MFC systems with a multi-anode/cathode configuration operated under continuous flow conditions. Stacking of multiple syngas-fed meMFCs sharing a common gas and influent passage as that demonstrated in this study might avoid the requirement of an extensive distribution system to pump the substrate gas and influent individually to different reactors as required for a stack of multiple single-anode/cathode systems (Ieropoulos et al., 2008; Zhuang andZhou, 2009), thereby providing easy construction and implementation of scale-up systems.

To confirm the impact of different anode-cathode arrangements on MFC power output, meMFC#2 was also operated under several external circuit connections as previously described in Table 6.1. Similar to tests with meMFC#1, reduction in number of electrically connected electrodes negatively impacted the power output of meMFC#2. However, the extent of impact was dependent on the operating conditions and microbial activity.

Similar to meMFC#1 tests, a reduction in power density in meMFC#2 on reducing the number of electrically connected cathodes was more profound under optimal operating conditions. A decrease in power density of up to 55 % upon cathode area reduction was observed for meMFC#2 at the optimal operating temperature of 37 °C and a syngas flow rate of 2 $L L_R^{-1} d^{-1}$. Compared to a power output of 33 $mW L_R^{-1}$ in a 3A-2C arrangement, a

reduced power density of 20 mW L_R^{-1} in 3A-1C(L) arrangement and 15 mW L_R^{-1} in 3A-1C(R) arrangement was obtained (Fig. 6.6). The R_{int} correspondingly increased to 36 Ω and 40 Ω, respectively, as compared to 24 Ω estimated in the polarization test for 3A-2C configuration. A substantial reduction in power output in 3A-1C arrangement was also observed under substrate limited conditions at a syngas flow rate of 1.2 L L_R^{-1} d^{-1}. The maximum power density of 12 mW L_R^{-1} in 3A-1C(L) arrangement and 10 mW L_R^{-1} in 3A-1C(R) arrangement were approximately 29 % and 41 % lesser than the maximum power density of 17 mW L_R^{-1} obtained in 3A-2C arrangement (Fig. 6.6).

A reduction in cathode surface area had a smaller impact on power output during meMFC#2 operation at 50 °C and 45 °C, where the system performance was limited by low biocatalytic activity. The meMFC#2 operation in the 3A-1C electrode arrangement at 50 °C and 45 °C resulted in up to 20 % decrease in power density (Fig. 6.7), as compared to a reduction of up to 55 % observed at 37 °C under a similar electrode arrangement. For example, the maximum power density of 9 mW L_R^{-1} in a 3A-1C(L) arrangement and 8 mW L_R^{-1} in 3A-1C(R) arrangement at 50 °C, were slightly lower to the power density of 10 mW L_R^{-1} obtained in 3A-2C arrangement (Fig 6.7). A similar observation was also made during meMFC#2 operation at the syngas flow rate of 3 L L_R^{-1} d^{-1}, where the high gas flow rate inhibited microbial activity. The maximum power density of 10 mW L_R^{-1} in 3A-1C(L) arrangement and 9 mW L_R^{-1} 3A-1C(R) were 17 and 24 % less than the maximum power density of 12 mW L_R^{-1} obtained in 3A-2C arrangement (Fig. 6.6).

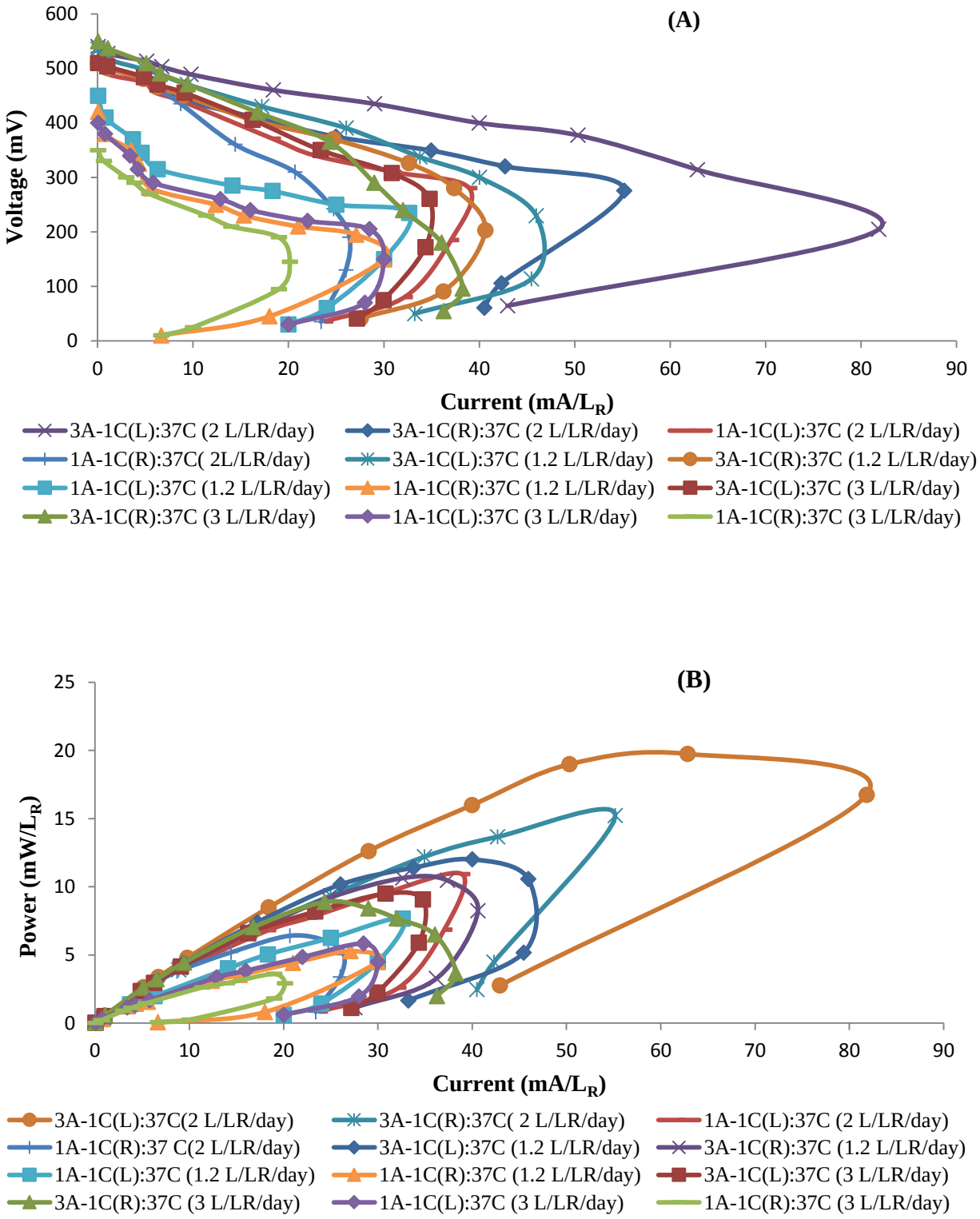

Figure 6.6 (A) Polarization and (B) power curves under different electrode arrangements and syngas flow rates for syngas-fed multi-anode/cathode MFC (meMFC#2) at the operating temperature of 37 °C.

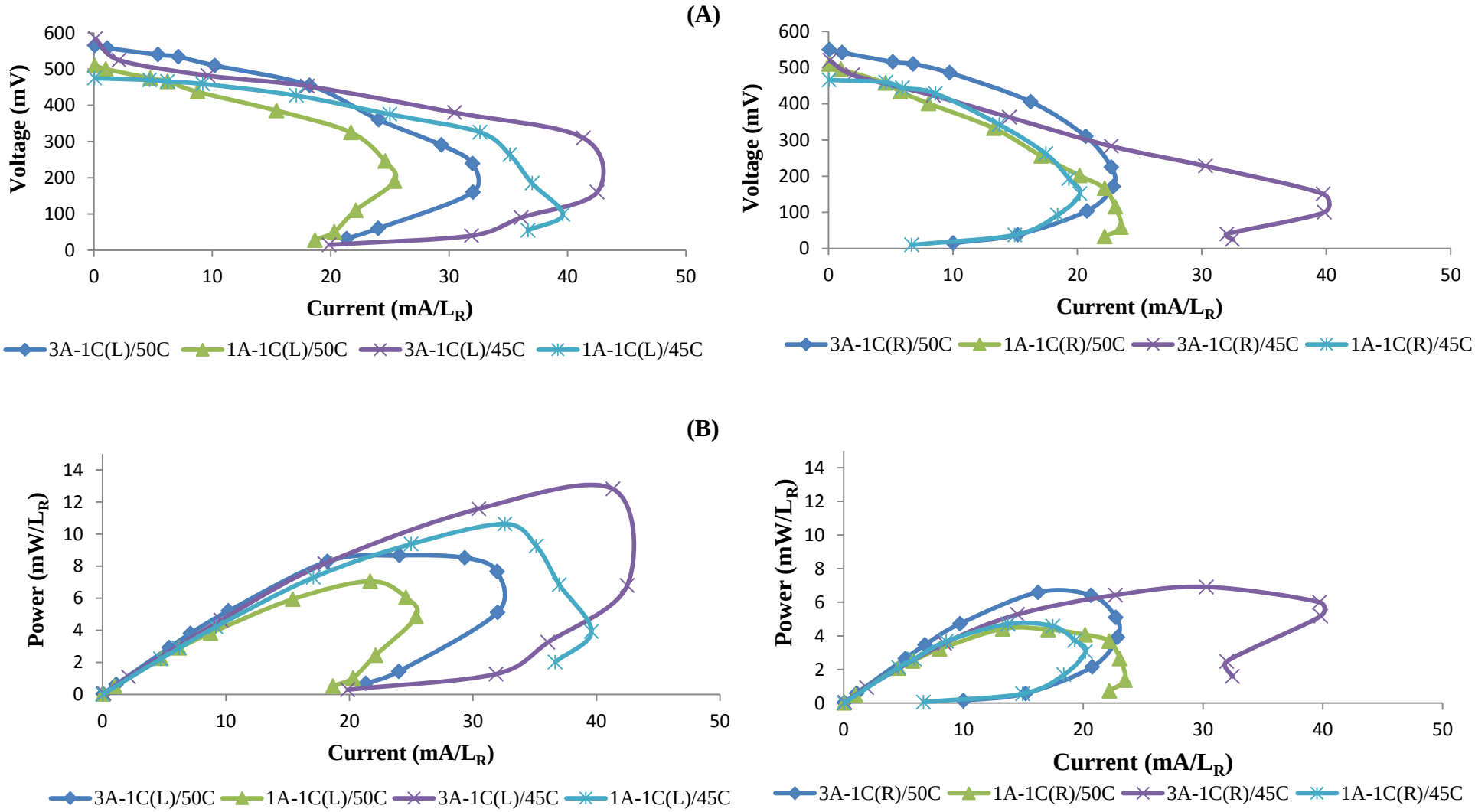

Figure 6.7 (A) Polarization and (B) power curves for syngas-fed multi-anode/cathode MFC (meMFC#2) under different electrode arrangement at operating temperatures of 45 °C and 50 °C.

Anode connection tests in meMFC#2 also confirmed the trends observed for meMFC#1, where a reduction in the number of electrically interconnected anodes resulted in a reduction in power densities of up to 46 % (Fig. 6.6). For instance, the power density of 11 $mW\,L_R^{-1}$ achieved in 1A-1C(L) arrangement at 37 °C and a syngas flow rate of 2 $L\,L_R^{-1}\,d^{-1}$, was ~ 45 % less as compared to the power output observed in 3A-1C(L) arrangement (20 $mW\,L_R^{-1}$). Similarly, the power density of 8 $mW\,L_R^{-1}$ achieved in 1A-1C(R) arrangement at 37 °C was ~ 46 % less as compared to the power density of 15 $mW\,L_R^{-1}$ achieved in 3A-1C(R) arrangement at similar flow rate (Fig 6.6).

The impact of anodic area reduction in meMFC#2 was slightly more pronounced at 45 °C and 50 °C. The meMFC#2 operation in 1A-1C(R) arrangement at 50 °C resulted in 50 % decrease in power density to 4 $mW\,L_R^{-1}$, as against 8 $mW\,L_R^{-1}$ obtained in 3A-1C(R) arrangement (Fig.6.7). Similarly, at the operating temperature of 45 °C, the power density of 5 $mW\,L_R^{-1}$ in 1A-1C(R) arrangement was ~ 55 % lower than the power output of 12 $mW\,L_R^{-1}$ achieved in 3A-1C(R) arrangement (Fig 6.7). The reduction in power density was even higher during meMFC#2 operation at 37 °C at a syngas flow rate of 3 $L\,L_R^{-1}\,d^{-1}$, where the bioelectrocatalytic activity was inhibited by the high gas flow rate. The maximum power density of 3 $mW\,L_R^{-1}$ obtained in 1A-1C(R) arrangement was ~ 63 % lower than the power output of 9 $mW\,L_R^{-1}$ achieved in 3A-1C(R) arrangement (Fig 6.6).

At the end of the electrode arrangement tests and meMFC#2 operation, test samples were drawn from each carbon felt anode for protein analysis. A protein concentration of 0.16 mgL_A^{-1} for anode A1, 0.12 mgL_A^{-1} for A2 and 0.06 mgL_A^{-1} for A3 were obtained. Assuming that the protein concentration is proportional to the anodic biofilm density (Gil-Carrera et al., 2011), it can be inferred that the biofilm growth was highest at anodes (A1, A2) closest to the cathodes, while the increased distance from the cathodes might have restricted biofilm growth at anode A3. A reduction in anodic biofilm formation and growth upon the increased distance from the cathode has been previously reported (Gil et al., 2003). This dependence is related to an increased resistance to charge transport with increased electrode distances which limits the growth of anodic microorganisms. This implies that the performance of meMFC#2 can be further enhanced by improvements in system design and hydraulic conditions to minimize charge transport resistance.

Several reasons can be cited to explain the enhanced efficiency of the multi-anode/cathode configuration used in this study. First, the incorporation of multiple anodes allowed for increased anodic packing (total volume of anodes to the total volume of the anodic compartment). The anodic packing density of 0.75 (75 mL anode/ 100 mL anodic compartment) for meMFC #2 was 25 % higher than the anode packing density of 0.5 (25 mL anode/50 mL anodic chamber) for syngas-fed single anode/cathode MFCs in our previous studies (Hussain et al., 2011b; Mehta et al., 2010). An enhanced power density and CE with increased anodic packing due to the availability of a larger surface area for biofilm attachment and development has been widely reported (Logan, 2008). However, an increased anodic volume in a single-cathode configuration also leads to an increased R_{int} due

to a greater distance to the cathode, and consequently a greater mass and charge transport resistance (Logan, 2008). Such transport limitations may have been reduced by the two-cathode arrangement used in this study, consequently resulting in higher power outputs.

The improved performance of meMFC#2, at least at mesophilic temperatures, also suggests that the power density up to a certain level can be enhanced by increasing the number of cathodes while keeping the total cathode area constant. Notably, the cathode specific area of 0.1 $m^2 L_R^{-1}$ (geometric cathode area of 100 cm^2/ total anodic compartment of 100 mL) for meMFC#2 was equal to the cathode specific area obtained for syngas-fed single anode/cathode MFCs in our previous studies (geometric cathode area of 50 cm^2/ 50 mL anodic compartment). This observation is consistent with the study of Jian et al.(2010), where it was demonstrated that for increasing the power production of an MFC increasing the number of cathodes was more effective than increasing the cathodic surface area. The same study elucidated that for MFCs with similar volumes and dimensions, a 2-anode/cathode configuration with a total cathode area of 39 cm^2 produced a considerably higher power output of 159 mW/m^3 compared to 64 mW/m^3 obtained for a single-anode/cathode configuration with an equal cathode area (39 cm^2). Moreover, doubling the cathode area to 79 cm^2 with the single-anode/cathode configuration marginally increased the power output to 87 mW/m^3. It was suggested that increasing the number of cathodes allows the electrons generated at the multiple anodes in a multi-anode/cathode configuration to achieve much higher oxidation rates at the multiple cathodes, while limiting charge, mass and ohmic losses associated with the increased cathode area (Jiang et al., 2010). This phenomenon of improved power generation with an increase in the number of cathodes

while keeping the total cathode area constant could have considerable economic and operational benefits for large scale MFC systems, especially considering that the high cost of cathodes is one of the major factors governing the economic feasibility of the MFC systems.

The meMFC#2 performance at 50 °C was limited by the low thermophilic potential of the inoculum, which was further substantiated by the steady increase in performance in terms of power density and CE upon the reduction of the operating temperature from 50 °C to 37 °C. A drastic decline in power production at temperatures of 50 °C in MFCs inoculated with mesophilic sludge having a low thermophilic potential has been previously reported (Behera et al., 2011; Liu et al., 2011). These observations provide good evidence that for enhanced performance at thermophilic conditions, an efficient MFC design needs to be well complemented with an optimized microbial consortium. Low power production due to biological constraints even after the optimization of the system architecture and design for thermophilic operations has also been reported (Carver et al., 2011). The microbiological activity and consequently the performance of syngas-fed meMFC at thermophilic temperatures could be improved by suitable microbial adaptation, selection and enrichment procedures or by directly utilizing inocula obtained from thermophilic anaerobic digesters with conceivably high thermophilic potential. In this case, much higher power densities can be expected due to the increased biocatalytic activity and electrochemical reaction rates at elevated temperatures within a certain range.

Significant methane production was observed throughout meMFC#2 operation (Table 6.2). Methanogens compete with the electricigenic microorganisms for the electron

donor and thereby negatively influence the Coulombic Efficiency. Similar observations were made in our previous studies (Hussain et al., 2011b; Mehta et al., 2010) and also in the study of Kim et al.(2005), where a 30 % increase in CE was obtained upon the utilization of a methanogenic inhibitor. Notably, meMFC#2 was operated with a hydraulic retention time of 5 days for improved biomass retention; this however, might have led to the retention and proliferation of methanogens in the anodic liquid. This implies that the CE can be further improved by the optimization of the influent flow rate to find a right balance between the retention of the intermediates utilized for electricity production and the removal of the planktonic methanogenic microorganisms. Methanogenesis could also be curtailed by the development of an MFC with an engineered microbial consortium. A syngas-fed meMFC seeded with pure cultures of CO-tolerant electricigenic microorganisms such as *Geobacter sulfurreducens* (for mesophilic operation) or *Thermincola ferriacetica* (for thermophilic operation), in a syntrophic relationship with carboxydotrophic and homoacetogenic microorganisms might be capable of achieving higher power densities and correspondingly an improved CE. A Coulombic Efficiency of ~ 97 % for a thermophilic acetate-fed MFC inoculated with a pure culture of *T. ferriacetica* has been recently reported (Marshall and May, 2009). Similarly, acetate-fed MFCs inoculated with an axenic culture of *G. sufurreducens* have been reported to achieve high power densities that were comparable to the MFCs inoculated with anaerobic sludge (Nevin et al., 2008).

6.5 Conclusion

This study demonstrates electricity generation in a multi-anode/cathode MFC fed with syngas, and systematically elucidates the impact of the operating temperature and electrode arrangement on system performance. The adoption of a multi-electrode design effectively enhanced the performance of syngas-fed MFC with a small operational space requirement. The power output of 33 mW L_R^{-1} and CE of 43 % obtained at 37 °C in this study, is the highest observed for any mesophilic syngas-fed MFC. The MFC performance at 50 °C was restricted by the low biocatalytic activity, which implies that for an enhanced system performance under thermophilic conditions an efficient system design needs to be well complemented with an optimized biological system.

The MFC performance was greatly affected by the electrode arrangement, with the highest power being achieved in a 3A-2C electrode arrangement. A reduction in power density upon reducing the number of electrically connected cathodes was more pronounced under optimal operating conditions, whereas reducing the number of anodes had a much greater impact under conditions limited by microbial activity.

An improvement in the anodic design, hydraulic conditions and biological system to restrict methanogenesis and mass and charge transport related resistance may further enhance the performance of a syngas-fed multi-anode/cathode MFC. Also, to our knowledge, this is the first study where MnO_2 based cathodes were used in a syngas-fed MFC. The absence of any visible CO-related inhibition of the MnO_2 cathode suggests that this non-noble cathode can be utilized to reduce the MFC construction costs.

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CHAPTER 7

General Summary, Novelty and Recommendations for future Research

Carbon monoxide and synthesis gas conversion to electricity in an MFC represents an ingenious yet simple technology for sustainable energy production from biomass which promises high transformation efficiency. Besides low environmental impact and efficient utilization of indigenous resources, CO/syngas conversion to electricity in an MFC may offer several advantages including higher durability, ease of operation and maintenance, and an effective platform for decentralized electricity production which could have major impact on the socio-economic development of the rural sectors across the globe. CO/syngas fed-MFCs are currently at an early developmental stage with only two exploratory studies been conducted prior to this thesis. A number of challenges pertaining to microbiology, electrochemistry, mass transfer and reactor design need to be addressed before the full potential of this technology can be realised. The studies performed in this thesis addressed two such challenges; first, the improvements in the reactor design and operating conditions to enhance gas-liquid mass transfer and system performance on CO/syngas, and second, the understanding of the biochemical pathways and microbiology driving electricity generation in a CO/syngas-fed MFC.

In this thesis, Chapter 1 and Chapter 2 (Hussain et al., 2011a), introduced the concept of electricity production from CO/syngas in an MFC and elaborated on the principles governing MFC operation. Various microbial communities including homo-

acetogens, acetogenic and hydrogenogenic carboxydotrophs, novel metal-reducing carboxydotrophs and other engineered consortia that could be utilized for efficient CO/syngas transformation and electricity generation were reviewed. Besides the microbiological consortium, the two chapters also reviewed and proposed various reactor designs and membrane systems suitable for improving MFC performance on CO/syngas and enhancing gas-liquid mass transfer. The ability of silicone membrane systems to efficiently transfer CO into the anodic liquid of an MFC was demonstrated in Chapter 3 (Hussain et al., 2011b). The use of flat silicone membrane system and thin wall silicone tubing efficiently enhanced the mass transfer with reduced reactor volumes, correspondingly resulting in enhanced CO transformation efficiency and improved volumetric power output.

The microbial communities enriched at the anode of two mesophilic CO-fed MFC and the biotransformation pathways involved in electricity generation were studied in Chapter 4. The identification of the microorganisms belonging to the genera *Geobacter, Desulfovibrio,* and *Clostridium,* along with the validated ability of pure cultures of *Geobacter sulfurreducens* to grow in 100 % CO atmosphere, affirmed that electricity production from CO/syngas in a mesophilic MFC dominantly takes place by a two-step process: CO is first converted to acetate, which is then oxidized by CO-tolerant electricigenic bacteria such as *G. sulfurreducens* for electricity production. A similar pathway of electricity production by a tight collaboration amongst interdependent and competing bacterial species collectively contributing to the consortium and catalyzing CO/syngas conversion to electricity was also observed at thermophilic temperatures. The analyses of the microbial communities and metabolic products of the syngas-fed MFC in Chapter 5 (Hussain et al., 2012), indicated the

existence of several concurrent pathways leading to electricity production under thermophilic conditions. Similar to the observations at mesophilic temperatures, electricity production under thermophilic conditions also involved syngas conversion to acetate by thermophilic homo-acetogens and acetogenic carboxydotrophs, followed by acetate oxidation by CO-tolerant electricigenic microorganisms. Efficient syngas transfer into the anodic liquid with the utilization of silicone membranes, and increased microbial catalytic activity at thermophilic conditions led to much higher power density, Coulombic efficiency and improved syngas transformation efficiency as compared to the mesophilic syngas-fed MFC in Chapter 3.

Finally in Chapter 6, the MFC design was further improved by the development of a multi-anode/cathode MFC comprised of three anodes and two cathodes. The ability of the multi-electrode design to achieve high volumetric efficiencies on syngas was examined at several operating temperatures and anode-cathode arrangements. Due to the improved design, the multi-electrode approach considerably increased the power output and Coulombic efficiency. The maximum power output and Coulombic efficiency was achieved under mesophilic conditions. The MFC performance at elevated temperatures was restricted by the low thermophilic potential of the mesophilic inoculum. MFC power output was also greatly impacted by the anode-cathode arrangement with the highest power output being achieved by electrically interconnecting all anodes and cathodes. Reduction in number of interconnected cathodes or/and anodes considerably decreased the power density. The extent of this decrease was found to be dependent upon the operating condition and microbial activity. Reduction in power density upon decreasing the number of interconnected cathodes

was more pronounced under optimal operating conditions, whereas anode reduction had a much greater impact under conditions limited by microbial activity.

7.1 Novelty and contribution to knowledge

This thesis presents the first detailed study of the microbiological interactions associated with CO/syngas conversion to electricity in an MFC and the reactor design improvements thereof. The outcome of this comprehensive study has contributed to our knowledge in several ways related to scientific and engineering/application challenges. The following are a few of the several commendable contributions of this research.

1. Low aqueous solubility of carbon monoxide and hydrogen is one of the major limiting factors in the efficient utilization and biotransformation of these gaseous substrates. The ability of relatively inexpensive silicone membrane systems to efficiently transfer CO/syngas into the anodic liquid of an MFC under mesophilic and thermophilic conditions offers a tangible solution to this engineering challenge, which may not only assist in the future development and optimization of CO/syngas-fed MFCs, but also in the microbiological conversion of CO/syngas to biofuels/bioproducts.
2. This study for the first time elucidated the microbial communities and biotransformation pathways involved in CO/syngas conversion to electricity under mesophilic and thermophilic conditions. Also, syngas dependent bioelectrochemical activity at thermophilic temperatures was demonstrated for the first time. Given that electricity production in an MFC is governed by biological activity, the results obtained in this study play a crucial role for understanding and optimization of the microbiological

process and the MFC performance using CO/syngas. In addition, the identified microbial communities may further assist in the understanding of carbon monoxide-dependent energy metabolism, microbe-electrode interactions and ecophysiological features of mesophilic and thermophilic homo-acetogens and carboxydotrophs.

3. We demonstrated the ability of pure cultures of *Geobacter sulfurreducens* to grow in 100 % CO atmosphere. This previously unknown physiology of *G. sulfurreducens*, may further assist in the optimization of the microbiological constortium of CO/syngas-fed MFCs. *G. sulfurreducens* have been widely utilized for electricity production in MFCs with high Coulombic efficiency. Axenic cultures of this microorganism in syntrophic relationship with homo-acetogens and carboxydotrophs may also result in increased power outputs and Coulombic efficiency on CO/syngas. The ability of *G. sulfurreducens* to grow in 100 % CO atmosphere may also contribute in the advancement of studies focusing on the utilization of this microorganism for environmental decontamination and desulfurization with CO/syngas serving as the electron donor.
4. This study also presented the development of several reactor designs including multi-anode/cathode MFC for improved performance on CO/syngas under mesophilic and thermophilic conditions. These reactor designs may be used in the future for continued enhancement of this technology, and may result in the development of stackable MFCs capable of efficient operation on CO/syngas and with power outputs suitable for commercial applications. The reactor configurations presented herein may also be extended for addressing reactor design issues for CO/syngas fermentation.

7.2 Recommendations for further research

This study presented the reactor designs that can be utilized for improving MFC performance on CO/syngas. In addition the complex microbial interactions and biotransformation pathways involved in electricity production from CO/syngas were also elucidated. Commercial applications of CO/syngas-fed MFCs would require much higher volumetric efficiencies, which in turn would require further analysis and optimization of process microbiology, operating conditions and reactor design. Some of the recommendations for future research are as follows:

1. Methanogenesis presents one of the major barriers to achieving high Coulombic efficiency and power output in CO/syngas-fed MFCs. Methanogens compete with the electricigens for electron donor and use electron acceptors which does not result in electricity production. Such alternative metabolism could be curtailed by the pre-treatment of the inocula and the optimization of the auxiliary operating conditions such as pH and hydraulic retention time. The optimization of the influent flow rate to find a right balance between the retention of the intermediates and wash out/removal of planktonic methanogenic microorganisms may result in higher power outputs and correspondingly higher Coulombic efficiency.
2. Alternative metabolisms that do not result in electricity production could also be limited by the development of an MFC with an engineered microbial consortium. Future studies could focus on the development of a CO/syngas-fed MFC seeded with pure cultures of CO-tolerant electricigenic microorganisms such as *G. sulfurreducens*, which in a

syntrophic relationship with pure cultures carboxydotrophic and homoacetogenic microorganisms might be capable of achieving higher power densities.

3. Future studies might focus on testing other gas diffusion membranes with a high affinity for CO and H_2 or the development of CO/syngas-fed columnar MFC reactors such as bubble column, trickle bed, and airlift reactors that could offer improved gas transport with small reactor volume. Improved gas transfer may also allow for focusing on the process microbiology, which could lead to the isolation of novel electricigenic carboxidotrophic microorganisms.
4. Further studies could also be focused on the investigation and analysis of recently isolated novel metal reducing carboxydotrophic microorganisms to generate electricity in a CO-fed MFC. Bacteria such as *Geobacter sulfurreducens, G. Metallireducens, Desulfobulbus propionicus* etc, capable of metal reduction have been widely used to produce electricity in an acetate and waste water-fed MFC with high Coulombic efficiency. Metal reducing microorganisms such as *Thermosinus carboxydivorans, Carboxydothermus ferrireducens, Thermincola ferriacetica,* etc. which are capable of utilizing Fe(III) as the final electron acceptor with CO serving as the sole source of energy, may also be capable of generating electricity in a CO-fed MFC with high Coulombic efficiency.
5. The task of process and design optimization of a CO/syngas-fed MFC could also be facilitated by process modelling. Development of bio-electrochemical models analyzing/describing anodic biofilm formation, species distribution and competition among methogenic, carboxydotrophic, and electricigenic microorganisms may allow for

a deeper understanding of MFC operation on CO/syngas and assist in identifying and circumventing major limitations.

6. Practical applications of CO/syngas-fed MFCs would conceivably require the stacking of multiple large-scale MFC systems with a multi-anode/cathode configuration operated under continuous flow conditions. Future research could be focused on developing CO/syngas-fed stack configurations capable of achieving high volumetric efficiencies. Proposed design for a fluidically bridged stack comprising two individual multi-electrode MFCs is provided in Fig. 7.1. The MFCs in the stack configuration may share a common gas and influent passage as shown in the figure below. This design might help to avoid the requirement of an extensive distribution system to pump the substrate gas and influent individually to different reactors and thereby allowing easy construction and implementation of scale-up systems.

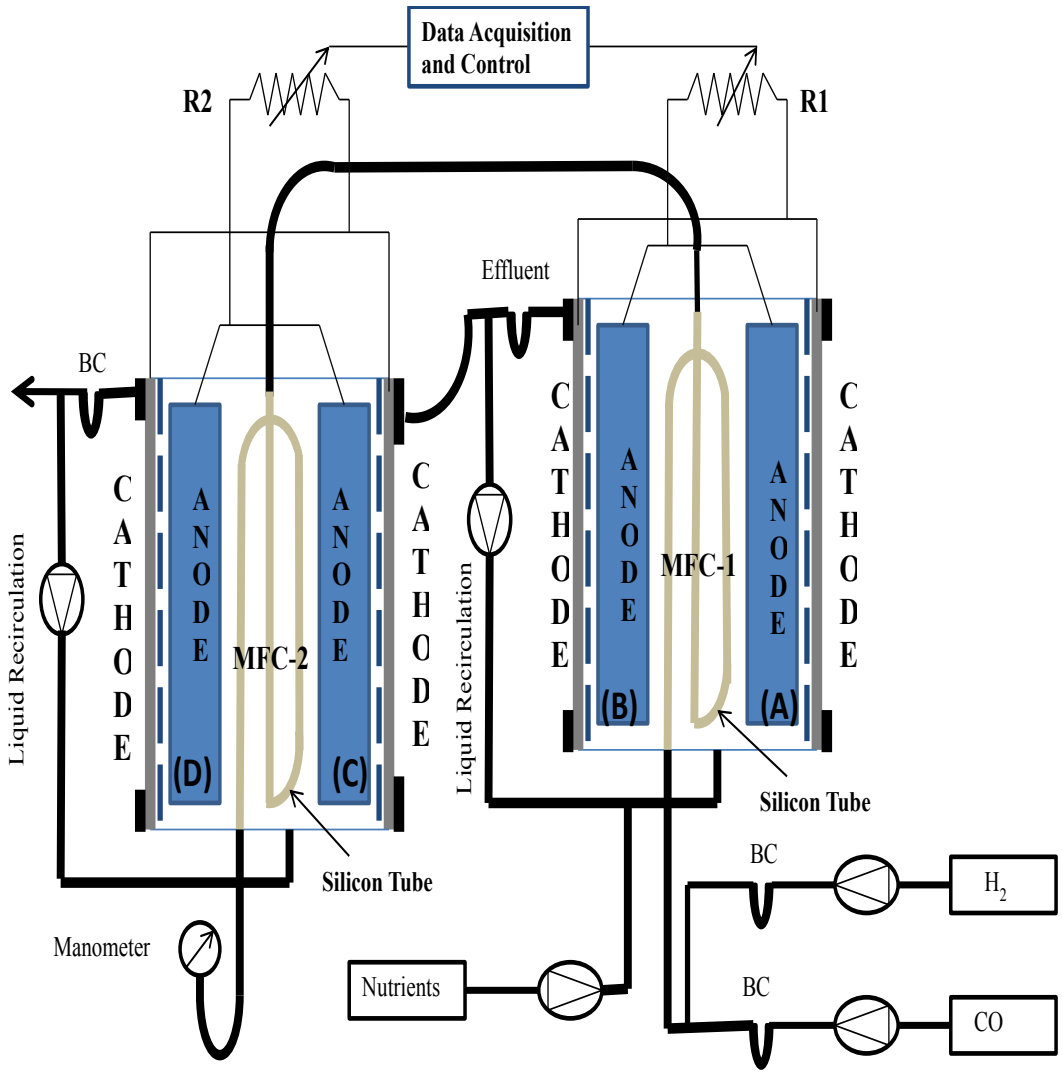

Figure 7.1 Proposed experimental design of a two MFC stack configuration for operation on CO/syngas.

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APPENDIX A

The entire experimental work for this thesis was performed at the Bioenegineering Facility at the Biotechnology Research Institute, National Research Council of Canada (NRC), 6100 Royal Mount Avenue, Montreal, Quebec, Canada. All the appropriate ethics, and biosafety guidelines and regulations were carefully followed.