ISTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

EFFECTS OF VARIOUS CARBOHYDRATES ON ELECTRICITY GENERATION IN MICROBIAL FUEL CELLS

Ph.D. Thesis by
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Department : Advanced Technologies
Programme: Molecular Biology-Genetics and Biotechnology

AUGUST 2008

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Date of submission : 13 June 2008

Date of defence examination: 05 August 2008

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AUGUST 2008

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

ÇEŞİTLİ KARBONHİDRATLARIN MİKROBİYAL YAKIT HÜCRELERİNDE ELEKTRİK ÜRETİMİNE ETKİLERİ

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Tezin Enstitüye Verildiği Tarih : 13 Haziran 2008
Tezin Savunulduğu Tarih : 05 Ağustos 2008

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AĞUSTOS 2008

ACKNOWLEDGMENTS

I am very thankful to my advisor, Dr. Hakan Bermek for both his support and trust on me during PhD program. In the summer of 2006, I fortunately started to work with my co-advisor, Dr. Kaichang Li. His contribution and mentorship not only to my PhD work, but also to my scientific career is definitely unforgettable, and I will always be thankful to him. Now, I feel very lucky because I had a chance to work with Dr. Hong Liu. I sincerely thank Dr. Liu for her encouragement, guidance and advisory during my PhD research. I sincerely thank Dr. Yanzhen Fan for his help in instrument calibration, microbial fuel cell construction, and his comments during my PhD research. I collectively thank the people I mentioned above for making me feel not only as a part of the research team, but also part of a family.

I thank Hongqiang Hu, who was my laboratory co-worker, for his help and support during my laboratory work. I thank another laboratory co-worker, Shoutao Xu who performed polymerase chain reaction and denaturing gradient gel electrophoresis in microbial community analysis. I also thank the following people (in alphabetical order) for their help, nice friendship during my PhD research: Chontisa Sukkasem, Dr. Ganti Murthy, Evan Sharbrough, Jian Huang, Katie Zhou, Michelle Yankus, Pınar Hüner, Wen Bai and Yudith Nieto.

I, of course, thank the thesis follow-up committee members, Prof. Melek Tüter, Dr. Ayten Yazgan Karataş and Dr. Alper T. Akarsubaşı for their suggestions and help. I also thank Turkish State Planning Organization and Oregon State University research fund.

I am also very thankful to my family for their love, and great support to my life.

Finally, I thank collectively everyone I mentioned above, and many others for their help in making my thesis like this possible.

June-2008 **Tunç Çatal**

CONTENTS

ABBREVIATIONS

BOD	: Biological oxygen demand
COD	: Chemical oxygen demand
DGGE	: Denaturing gradient gel electrophoresis
GC	: Gas chromotography
HMF	: Hydroxy-methyl furaldehyde
HPLC	: High pressure liquid chromotography
MFC(s)	: Microbial fuel cell(s)
PCR	**:** Polymerase Chain Reaction
SEM	: Scanning electron microscopy
SPSS	: Statistical Package for the Social Sciences
VFA	: Volatile fatty acids

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EFFECTS OF VARIOUS CARBOHYDRATES ON ELECTRICITY GENERATION IN MICROBIAL FUEL CELL

SUMMARY

In this study, the direct production of electricity from monosaccharides, disaccharides and sugar alcohols were examined using air cathode microbial fuel cells (MFCs). Electricity was produced from all substrates tested. The mixed bacterial culture enriched using sodium acetate as a carbon source adapted well to all carbon sources tested. The adaptation time varied for each substrate. Maximum power density obtained from the carbohydrates were in the range of 1262±5 mW m^{-2} and 2763±38 mW m^{-2}. For all substrates tested, the maximum voltage output at 120 Ω external resistance initially increased with the substrate concentration; however, further increases above a certain level did not improve the electricity generation. Coulombic efficiency was 10% to 34% for the compounds tested. For carbohydrates tested, the relationship between the maximum voltage output and the substrate concentration appeared to follow saturation kinetics at 120 Ω external resistance. Chemical oxygen demand removal was over 71% for all substrates tested. Two furan-derivatives and eight phenolic compounds were also examined, and 2-furaldehyde, acetophenone and 3-4-dimethoxybenzyl alcohol were found as strong inhibitors on voltage generation. Our results show that sulfuric acid hydrolysation (10%) of pine wood flour generate electricity in MFCs. Various sugar mixtures were preferentially used by the microorganisms, and carboxylic acids were produced as byproducts. Microbial community on biofilm structure was significantly affected by carbon source. Effect of pH on electricity production was examined, and was found as a significant factor on voltage. Results from this study indicated that lignocellulosic biomass-derived compounds might be a suitable resource for electricity generation using MFC technology.

ÇEŞİTLİ KARBONHİDRATLARIN MİKROBİYAL YAKIT HÜCRELERİNDE ELEKTRİK ÜRETİMİNE ETKİLERİ

ÖZET

Bu çalışmada, lignoselülozik biyokütlelerin asit hidrolizatlarında yaygın olarak bulunan monosakkaritlerden, disakkaritlerden, şeker alkollerinden direkt olarak elektrik üretimi, hava-katot mikrobiyal yakıt hücreleri kullanılarak araştırılmıştır. Başlıca on iki monosakkariti, iki disakkariti ve altı şeker alkollerini kapsayan karbon kaynakları ile elektrik üretimi gözlenmiştir. Sodyum asetat ile zenginleştirilmiş karışık bakteri kültürü, test edilen bütün substratlara kolayca adapte olmuştur. Yeni karbon kaynağına adaptasyon için gerekli süre substralar için farklılık göstermiştir. Test edilen substratlar için elde edilen en yüksek güç yoğunluğu 1262±5 mW m^{-2} ve 2763±38 mW m^{-2} arasında bulunmuştur. Kolombik yeterlik yüzde 10-34 idi. Test edilen substratlar için, en yüksek volt eldesi ve substrat konsantrasyonu arasında ilişki 120 ohm dış dirençte doygunluk kinetiği sonuçları ile uyumlu olduğu görülmüştür. Test edilen karbonhidratlar için yüzde 71'nin üzerinde kimyasal oksijen talebinde azalma sağlanmıştır. İki furan türevi ve sekiz fenolik bileşiğin araştırılması yapılmış, 2-furaldehit, asetofenon ve 3-4-dimetoksibenzil alkol'ün voltaj üretimi üzerine güçlü inhibitor etki gösterdiği saptanmıştır. Test edilen çam odun tozu hidrolizatının elektrik üretiminde karbon kaynağı olarak kullanılabileceği keşfedilmiştir. Monosakkarit karışımlarının mikrobiyal yakıt hücrelerinde tercihli kullanılarak elektrik üretimine yol açtığı ve karboksilik asit olarak yan ürünler oluşturulduğu saptanmıştır. Çalışmada ayrıca çeşitli operasyonel parametrelerin araştırılması yapılmıştır. Karbon kaynaklarının biofilm üzerinde mikrobiyal çeşitliliği önemli olarak etkilediği saptanmıştır. Çalışmada ayrıca, elektrik üretimi üzerine pH etkisi araştırılmış ve önemli bir faktör olduğu bulunmuştur. Bu çalışmanın sonuçları, lignoselülozik maddelerden türevli monosakkaritlerin, disakkaritlerin, şeker alkollerinin ve odun türevli maddelerin ön muamele ile mikrobiyal yakıt hücreleri için uygun birer karbon kaynağı olabileceklerini göstermiştir.

1. INTRODUCTION

1.1 Energy Needs, Green-alternatives

Energy needs have been rising in recent years because of the increase in world population, and the increase consumption of energy resources. For example, in Turkey, the electric energy need is expected to rise to 1.173 billion kWh in 2050 requiring 360 billion kWh alternative energy (Yumurtaci and Asmaz, 2004; Ediger and Akar, 2007). Oil is mainly consumed to provide energy, however this brings many problems such as reserve limitation and global climate change/warming. In this respect, energy generation from renewable resources might have great potential to provide energy needs in a sustainable and environmentally-green manner in order to reduce the dependence on fossil fuels. Several renewable energy technologies have been reported, for example, solar power, wind power, hydroelectricity (Yumurtaci and Asmaz, 2004), biofuels (such as biethanols, biodiesel, biohydrogen) and biomass. Each technology has its own advantages and disadvantages, and green electricity is one of the alternatives. As a general definition, renewable electricity is described as the generation of electricity from renewable resources such as sunlight, wind, etc., and is pointed as green energy.

There are several types of lignocellulosic biomass in the world such as crops, woods, and their residues. Mainly, lignocellulosic biomass comes from forestry and agricultural activities. Up to now, many approaches have been reported for energy production purposes (Petrus and Noordermeer, 2006). For example, biohydrogen, bioethanol and biodiesel generation from renewable resources have been previously reported (Oh *et al.,* 2005; Ohgren *et al.,* 2006; Kerstter and Lyons, 2001). A variety of methods are available for converting lignocellulosic biomass to energy (Cantarella et al., 2004). These processes convert biomass into a variety of gaseous, liquid, or solid fuels that can then be used directly in a power plant for energy generation. The carbohydrates in biomass, which are comprised of oxygen, carbon, and hydrogen,

can be broken down into a variety of chemicals, some of which are useful fuels. This conversion can be done by (1) thermochemical (by heating, and mediating of biomass gasifiers), (2) biochemical (by microorganisms, or enzymes), and (3) chemical (chemical convertion) ways.

Recently, energy generation by microbial fuel cell (MFC) technology received great attention because of recent significant improvements, and this approach/method has been reported as a promising technology for environmentally green energy generation (Logan and Regan, 2006).

1.2 Microbial Fuel Cell Technology

1.2.1 Brief History

The relationship between biology and electricity was discovered by Luigi Galvani in 1791 inventing the generation of electricity by muscle and nerve cells in frog legs. His invention also led to the discovery of primitive battery by Alessandro Volta who developed the first electric cell in 1800. The principle of fuel cells was published by Christian Friedrich Schönbein in 1839, and the first fuel cell was developed by William Robert Grove in 1845. Michael C. Potter studied electricity generation by MFCs in 1912 using *E. coli* (Potter, 1912). Later, Barnet Cohen demonstrated that a number of half-MFCs connected in series produced over 35 volt (with 2 mA) (Cohen, 1931). In early 1980's, Peter Bennetto's work helped to understand the priciples of microbial fuel cell operation (Bennetto *et al.,* 1983). During the improvement of MFC technology, this method was also suggested for biosensor applications, especially for removal of biological oxygen demand by Byung Hong Kim (Kim *et al.,* 2003).

1.2.2 Principle of a microbial fuel cell operation

Typically, MFCs use bacteria which catalyze the conversion of organic matter into electricity by attaching onto electrode surface area forming a biofilm which is common for microbial communities in MFCs (Liu *et al.,* 2004: Kim *et al.,* 2006). The properties of the biofilm and its features are determined by the microbial community (Sutherland, 2001). Once produced by microorganisms, electrons flow

from anode to cathode creating a current. Bacteria grow by catalyzing chemical reactions and store energy in the form of ATP. However, some bacteria oxidize reduced substrates and transfer electrons to respiratory-chain enzymes by NADH (the reduced form of nicotinamide adenine dinucleotide). These electrons flow down a respiratory chain—a series of enzymes that function to move protons across an internal membrane—creating a proton gradient. The protons flow back into the cell through the enzyme ATPase, and create 1 ATP molecule from 1 ADP for every 3–4 protons. Finally, the electrons are released to a soluble terminal electron acceptor, such as nitrate, sulfate, or oxygen (Logan and Regan, 2006).

1.2.3 Electron transfer mechanisms and microbial fuel cell configurations

MFCs can be divided into two types depending on how electrons are transferred from bacteria to the anode. At the same time, depending on the microorganism species, electron transfer mechanisms may vary, and also determine the type of MFCs if they are (1) mediated or (2) mediator-less (Fig. 1.1). Up to now, three different electron transfer mechanisms from microorganisms have been reported: (1) bacterial nanowires, (2) electron transfer by cell-surface proteins, and (3) chemical mediators. The role of bacterial nanowires in electron transfer has been demonstrated in *Shewanella* species (Gorby *et al.,* 2006). It was shown that *Geobacter sulfurreducens* outer-membrane cytochromes, might play a role in electron transfer from microorganisms to electrodes (Magnuson 2001). The direct electron transfer from yeast cells were demonstrated in mediator-less MFCs (Prasad *et al.,* 2007). Zhang *et al.,* indicated that electron transfer between electrode and *E. coli* cells is carried out by soluble compounds in the culture (Zhang *et al.,* 2008). On the other hand, *Pseudomonas aeruginosa* has been reported as a mediator producer, that is phenazine, to stimulate electron transfer for several bacterial strains (Rabaey, 2004). Another, exoelectrogenic bacterium *Ochrobactrum anthropi* has been reported, recently (Zuo *et al.,* 2008).

Mediated MFCs require electron shuttlers that are generally toxic compounds, and must be replaced during continuous operation (Cheng *et al.,* 2006). On the other hand, in recent years, single chamber mediator-less MFCs have been introduced, and they do not require exogenous chemicals to provide electron transfer to the electrode (Liu and Logan, 2004).

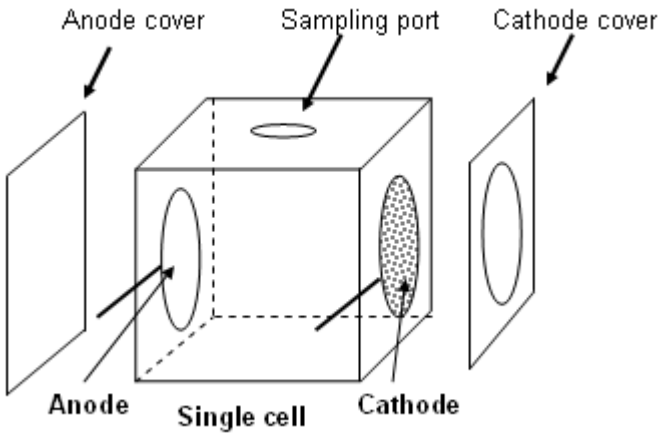

Figure 1.1: A diagram of an air cathode, single chamber, mediator-less MFC

1.2.4 Evaluation of an MFC performance

Typically, the first step in the evaluation of a MFC is the measurement of circuit voltage using a multimeter, or a data acquisition system. The voltage is a function of the resistance, or load on the circuit, and the current (*I*), which can be calculated using voltage based on Ohm's law ($E=I.R$). Ohm's law defines the relationships between (*P*) power, (*E*) voltage, (*I*) current, and (*R*) resistance. One ohm is the resistance value through which one volt will maintain a current of one ampere as seen in the equation below. Power density is another parameter to evaluate MFC performance. The power output (*P*) of an MFC can be calculated according to $P = IV$. Power output is often normalized to the projected electrode surface area, or calculated based on the volume of a MFC. Current density is used as a function of power density curves. Coulombic efficiency is used to evaluate the electron recovery given to the system as current from the organic substance. Coulombic efficiency is calculated as a ratio of total recovered coulombs obtained by integrating the current over time to the theoretical coulombs that can be produced from the substrate. Energy recovery is used to compare the performance of MFCs, and is the ratio of power produced by the cell to the theoretical heat energy of the organic substrates added. Chemical or biological oxygen demand (COD; BOD) can be removed in MFCs through conversion to electrical current, biomass, and/or through sulfate and nitrate reduction, or micro-aerobic oxidation, and be used to evaluate the MFC performance together with other parameters (Logan and Regan, 2006).

1.2.5 Materials for microbial fuel cells

Material selection/development for sustainable MFC applications is very important to achieve higher power output and Coulombic efficiency as well as lower cost. Mainly, MFCs consist of two electrodes, anode and cathode. In anode microorganisms play a catalyst role, while in cathode, chemical catalyst is required, therefore, improvement of novel catalysts is another challenge in MFC research. A good anode material should be (1) good in conductivity, (2) sustainable, (3) cheap, and (4) provide enough surface area for microorganisms (Logan and Regan, 2006). Up to now, several materials have been reported, and mainly carbon cloth is used (Liu and Logan, 2004). Heijne *et al.* (2008) compared four non-porous materials for their suitability as bio-anode in microbial fuel cells (MFCs). These materials were flat graphite, roughened graphite, Pt-coated titanium, and uncoated titanium (Heijne *et al.,* 2008). Qiao *et al.* (2007) evaluated a carbon nanotube/polyaniline composite as anode material for MFCs, and suggested the composite anode is excellent and is promising for MFC applications (Qiao *et al.,* 2007). Scott *et al.* (2007) have examined the effect of different carbon anodes in a single chamber MFC, and reported that the best performing anodes were made from carbon modified with quinone/quinoid groups (Scott *et al.,* 2007). Stainless steel was recently studied as anode for the biocatalysis of acetate oxidation by biofilms of *Geobacter sulfurreducens* (Dumas *et al.,* 2008). You *et al.* (2007) developed a graphite-granule anode, tubular air-cathode MFC capable of continuous electricity generation from glucose-based substrates, and suggested a feasible and simple method to reduce internal resistance and improve power generation of sustainable air-cathode MFCs (You *et al.,* 2007).

Development of suitable catalysts for MFCs is very popular research area. Moris *et al.* (2007) have compared lead dioxide (PbO_2) and platinum (Pt) as cathode catalysts in a double-cell microbial fuel cell (MFC) utilizing glucose as a substrate in the anode chamber, and suggested that cathode designs that incorporate PbO_2 instead of Pt could possibly improve the feasibility of scaling up of MFC designs (Moris *et al.,* 2007). HaoYou *et al.* (2007) examined various cathode catalysts prepared from metal porphyrines and phthalocyanines for oxygen reduction activity in neutral pH media, indicating that MFCs with low cost metal macrocycles catalysts is promising in further practical applications (HaoYou *et al.,* 2007). Air-cathode Single chamber

MFCs lacking a proton exchange membrane (PEM) might be promising for many practical applications due to their low operational cost, simple configuration and relative high power density (Fan *et al.,* 2007). One of the challenges for PEM-less MFC is that the Coulombic efficiency is much lower than those containing PEM (Fan *et al.,* 2007). They indicated that the Coulombic efficiency and power density of air-cathode MFCs can be improved significantly using an inexpensive cloth layer, which greatly increases the feasibility for the practical applications of MFCs (Fan *et al.,* 2007). Oxygen is the most sustainable electron acceptor currently available for MFC cathodes (Freguia *et al.,* 2007). Several materials and catalysts have previously been investigated in order to facilitate oxygen reduction at the cathode surface. Freguia *et al.* (2008) showed that significant stable currents can be delivered by using a non-catalyzed cathode made of granular graphite (power outputs up to 21 W m^{-3} as cathode total volume with acetate). They suggested that the presence of nanoscale pores on granular graphite provides a high surface area for oxygen reduction, demonstrating that microbial fuel cells can be operated efficiently using high surface graphite as cathode material (Freguia *et al.,* 2007). Rosenbaum *et al.* (2007) examined the properties of tungsten carbide as anodic electrocatalyst for MFC application. They showed that the electrocatalytic activity and chemical stability of tungsten carbide is excellent in acidic to pH neutral potassium chloride electrolyte solutions, whereas higher phosphate concentrations at neutral pH support oxidative degradation (Rosenbaum *et al.,* 2007).

Liu and Li (2007) examined the effects of biological factors (anode inoculum species, inoculum concentration), as well as non-biological factors (cathode electron acceptor and proton exchange material) on electricity production of a dual-chamber mediator-less MFC in fed-batch mode, and suggested that electricity production is more significantly influenced by cathode electron acceptor and proton exchange material, less affected by the inoculum species and inoculum amount (Liu and Li, 2007). Picioreanu *et al.* (2007) evaluated a computational model for MFCs based on redox mediators with several populations of suspended and attached biofilm microorganisms, and multiple dissolved chemical species (Picioreanu *et al.,* 2007). Increasing power densities in MFCs require reducing the internal resistance of the system, and methods are needed to control dissolved oxygen flux into the anode chamber in order to increase overall Coulombic efficiency (Min *et al.,* 2005). You *et*

al. (2006) reported that permanganate could be used as an effective cathodic electron acceptor for a MFC (You *et al.,* 2006). The kind of MFC also determines the aim of MFC technology for various applications.

1.2.6 Alternative microbial fuel cell applications (wastewater, bioremediation)

In correct configurations, MFCs can also be used for wastewater treatment. The operation for electricity production using wastewater and the scale-up of this technology need to deal with some problems such as the necessity of continuous running due to the impractical storage of raw material. However, bioreactor-based electricity production has been suggested as a novel approach for wastewater treatment, and reactor-type MFCs may be an interesting technology for sulfate removal from wastewater (Rabaey *et al.,* 2006). Rabaey *et al.* (2005) reported a novel MFC which anode part of the cell consists of granular graphite that permit wastewater to flow through the system and serve as surface area for bacteria to form biofilm structure. He *et al.* (2006) reported another kind of MFC in a tubular design where wastewater flows from the bottom to the top with continuous feeding of artificial wastewater containing sucrose solution. Neverthless, the power output achieved using MFCs were not high enough for a scale-up. Another problem is the requirement of removing dissolved oxygen from input wastewater material due to the inhibition effect on bacterial electricity generation (Liu and Logan, 2004). Mohan *et al.* (2008) evaluated the possibility of bioelectricity generation from anaerobic chemical wastewater treatment in dual-chambered; mediator-less anode, aerated cathode, plain graphite electrode MFC containing mixed cultures. They demonstrated the feasibility of *in situ* bioelectricity generation along with wastewater treatment, and the performance of MFC with respect to power generation and wastewater treatment was 731 mV at stable operating conditions (Mohan *et al.,* 2008).

MFC technology has also been suggested for bioremediation purposes. Kermanshahi pour *et al.* (2005) reported an immobilized cell airlift bioreactor for the aerobic bioremediation of simulated diesel fuel contaminated groundwater and tested with p-xylene and naphthalene in batch and continuous regimes, and suggested MFCs for successful bioremediation applications (Kermanshahi pour *et al.,* 2005).

1.3 The Aim of the Project

The aim of the current project is to evaluate of the possibility of lignocellulosic materials for electricity generation in single chamber, air-cathode, mediator-less MFCs. In this study, we first investigated the power generation in MFCs from various monosaccharides, disaccharides and sugar alcohols which are commonly found in lignocellulose-based hydrolysates. In order to assess the effect of potential inhibitor compounds that could arise from lignocellulosic material hydrolysis, we also investigated the effects of furan-derivatives and phenolic compounds, which were believed to be inhibitory on some microorganisms, thus, on electricity generation in MFCs. To better understand the substrate preference patterns of the cultures, sugar utilization patterns and generation of byproducts such as carboxylic acids during the utilization of sugars were examined. Operational parameters of an MFC such as pH were tested for future optimization studies, and effects of various sugars on microbial community were also investigated. Finally, we examined pine-wood flour acidic hydrolysate to understand the electricity generation profile from lignocellulosic materials based on the information obtained from the research above.

2. ELECTRICITY GENERATION IN MICROBIAL FUEL CELLS

2.1 Electricity Generation from Monosaccharides and Disaccharides

Recent efforts focused on finding renewable energy alternatives to fossil fuels. The production of fuel and energy from lignocellulosic biomass such as agricultural residues and woody biomass drew significant attention because of the abundance, ready availability and renewable nature of these resources (Petrus and Noordermeer, 2006; Ragauskas *et al.,* 2006). Main components of lignocellulosic biomass are cellulose, hemicelluloses and lignins. While cellulose is a homopolysaccharide consisting of D-glucose, hemicelluloses are branched heteropolysaccharides that are mainly composed of three hexoses (D-glucose, D-galactose, D-mannose), two pentoses (D-xylose and L-arabinose) and uronic acids such as galacturonic acid and glucuronic acid. Lignocellulosic biomass also contains a small amount of carbohydrates that are derived from the following monosaccharides: L-rhamnose, L-fructose, D-fucose, and D-ribose. Lignin is the most abundant aromatic polymer in nature, and is a complex polymer of phenylpropane units that are cross-linked to each other with a variety of different chemical bonds (Brigham *et al.,* 1996). Pre-treatment and subsequent hydrolysis of the lignocellulosic biomass into monosaccharides is often an essential processes for the production of biofuels such as ethanol and other biochemicals (Wiselogel *et al.,* 1996). The composition of the products obtained from pre-treatment and hydrolysis depends on the biomass sources as well as the pre-treatment/hydrolysis methods. Efficient utilization of all pre-treatment/hydrolysis products using relatively simple systems is indispensable for economic conversion of lignocellulosic biomass to energy and fuels (Petrus and Noordermeer, 2006; Hinman *et al.,* 1996).

MFC technology, which uses microorganisms to catalyze the direct generation of electricity from organic matter, provides a new method for the generation of renewable energy from biomass (Logan and Regan, 2006; Rabaey and Verstraete,

2005; Rezaei *et al.,* 2007). MFCs can use bacteria from natural environment to generate electricity from various substrates such as glucose, acetate, butyrate, lactate, ethanol, cysteine and bovine serum albumin as well as those from waste streams such as domestic wastewaters and various food industry wastewaters (Rabaey and Verstraete, 2005; Rezaei *et al.,* 2007; Liu and Logan 2004; Liu *et al*, 2005; Logan *et al.,* 2005). It was recently reported that hydrolysates from dilute acid pretreatment (1.2% w/v) of corn stover could be directly used in an MFC for electricity generation (Zuo *et al.,* 2006). The acid hydrolysates from pine wood or corn stover supposedly contain all monosaccharides previously described. However, whether all these monosaccharides can be utilized by bacteria in an MFC for electricity generation is poorly understood. The relative power generation capability of these monosaccharides is basically unknown.

In this study, we first investigated the power generation in MFCs from each of the 12 monosaccharides, including six hexoses (D-glucose, D-galactose, D(-)-levulose (fructose), L-fucose, L-rhamnose, and D-mannose), three pentoses (D-xylose, D(-)-arabinose, and D(-)-ribose), two uronic acids (D-galacturonic acid and D-glucuronic acid) and one aldonic acid (D-gluconic acid). Then, two different disaccharides, D-maltose and D-cellobiose were examined. We also investigated the Coulombic efficiency (E_c), the removal rate of chemical oxygen demand (COD) of the MFCs, the effects of monosaccharide and disaccharide concentration on the maximum voltage output and half-saturation constant.

2.1.1 Materials and experimental design

2.1.1.1 Microbial fuel cell construction

MFCs were constructed and modified as described previously (Liu *et al.,* 2005). The volume of a MFC chamber (made of plexiglass) was 12 mL with electrodes placed on the opposite sides of the chamber. Non-wet proofed carbon cloth (type A, E-TEK, Somerset, NJ, USA) and wet-proofed (30%) carbon cloth (type B, E-TEK Division, Inc., Somerset, NJ, USA) were used as anode and cathode, respectively. The air-facing side of the cathode was coated with carbon and poly(tetrafluoroethylene) (PTFE) layers, which was prepared according to a published procedure (Cheng *et al.,* 2006). The water-facing side of the cathode was coated with platinum (0.5 mg cm^{-2}

cathode area) using Nafion as a binder. For all the MFCs used in this study, the surface areas of the cathode and anode were 7.0 cm^2 and 2.0 cm^2, respectively.

2.1.1.2 Inoculation of a bacterial culture in a microbial fuel cell and the operation

Each of the twelve MFCs was inoculated with a mixed bacterial culture that was originally enriched from domestic wastewater and was maintained in our MFCs that had been operated for over one year using sodium acetate as carbon source. Sodium acetate (2000 mg L^{-1}) was initially used as the carbon source in each MFC, along with a medium solution containing: NH_4Cl (0.31 g L^{-1}); $NaH_2PO_4 \cdot H_2O$ (5.84 g L^{-1}); $Na_2HPO_4 \cdot 7H_2O$ (15.47 g L^{-1}); KCl (0.13 g L^{-1}), a mineral solution (12.5 mL) and a vitamin solution (12.5 mL) as described previously (Liu and Logan, 2004; Lovley and Phillips, 1988). The sodium acetate medium solution in each MFC was refreshed when the voltage decreased below 0.05 V. When a stable power output at 1k Ω was obtained, the sodium acetate solution was replaced with one of the following monosaccharides: 6.7 mM hexoses (D-glucose, D-galactose, D-mannose, D-fructose, L-fucose and L-rhamnose), 8 mM pentoses (D-xylose D-ribose and L-arabinose), 6.7 mM D-galacturonic acid, D-glucuronic acid, and D-gluconic acid. The following disaccahrides were also tested: D-mannose and D-cellobiose (3.33 mM). The different molar concentration of the monosaccharides and disaccharides was chosen in order to standardize the total carbon concentration in solution.

Polarization curves were prepared by varying the external resistance between 1k to 50 Ω. For each resistance, MFCs were ran for at least two batches to ensure repeatable power output could be achieved. Various concentrations of the monosaccharides and disaccharides (150-1400 mg L^{-1}) were also used to investigate the effects of the monosaccharide concentration on the electricity production at a fixed resistance of 120 Ω. Twelve MFCs with each containing different monosaccharides and disaccharides were run simultaneously in a constant temperature chamber (30 ±2 °C).

2.1.1.3 Analyses and calculations

Voltage outtput was measured using a multimeter with a data acquisition system (2700, Keithly, Cleveland, OH, USA). Power density (mW m^{-2}) was calculated according to $P=IV/A$, where I is the current, V voltage, and A the projected area of the anode. E_c is an important parameter in evaluating MFC performance and is described as the percentage of electrons recovered from the organic matter versus the theoretical maximum whereby all electrons are used for electricity production. The E_c was calculated as $E_c=C_P/C_{Ti}$ x 100%, where C_P is the total coulombs calculated by integrating the current over time, C_{Ti} is the theoretical amount of coulombs based on the added substrate.

Voltage was modeled as a function of substrate concentration (S) using Michaelis-Menten kinetics equation (formul 2.1) as follows:

$$V = \frac{V_{\max} S}{K_s + S} \tag{2.1}$$

where V_{max}, the maximum voltage and K_s (S), the half-saturation constant were determined using the Excel Solver (Microsoft, version 2003).

An aqueous sample taken from each MFC at the end of the batch experiment was filtered through a sterile syringe filter (0.22 μm). The filtrate was used for the determination of COD according to a standard method (American Public Health Association, 1992). Comparison of the planktonic bacterial concentrations in the MFC solutions was made by measuring the optical density (OD) at 600 nm using a spectrophotometer (UV-1700 Pharmaspec, Shimadzu, Japan).

2.2 Electricity Generation from Sugar Alcohols

MFCs, that use microorganisms rather than enzymes to breakdown organic materials and generate electricity, provide a new approach for renewable energy generation from biomass as mentioned earlier (Liu and Logan, 2004; Logan and Regan, 2006; Rabaey and Verstraete, 2005; Zuo *et al.,* 2006; Rezaie *et al.,* 2007; Davis and Higson, 2007; Catal *et al.,* 2008a). MFCs have also been investigated as biosensors

for monitoring lactate and biological oxygen demand (BOD) in wastewater (Karube *et al.,* 1977; Kim *et al.,* 1999; Chang *et al.,* 2004; Chang *et al.,* 2005; Kumlangham *et al.,* 2007). There are several factors affecting the performance of a MFC such as pH, external resistance, electrolyte as well as the type of substrate (Gil *et al*., 2003). It was reported that electricity could be generated from various organic materials, including sugars, such as monosaccharides (Catal *et al.,* 2008a; Liu and Logan, 2004), carboxylic acids, such as acetate, butyrate, propionate (Liu *et al.,* 2005), alcohols, such as ethanol, methanol (Kim *et al.,* 2007) proteins, such as bovine serum albumin (Heilmann and Logan, 2006), cellulose (Rezaie *et al.,* 2007), biomass hydrolysate (Zuo *et al.,* 2006), and wastewater streams (Rabaey, 2005). In recent years, significant improvements in the power generation by MFCs have been achieved. However, polyalcohols, which are important biomass-derived oxygenated feedstocks, have not been tested for electricity generation in MFCs although investigations has been made to produce clean fuel and hydrogen, from these polyalcohols via reformation (Chheda *et al.,* 2007).

In this part of the study, direct electricity generation from six polyalcohols, including three pentitols, namely xylitol, arabitol, ribitol, and three hexitols, namely galactitol, mannitol and sorbitol was demonstrated using single chamber air-cathode MFCs. The performance of the MFCs was evaluated on the basis of power density, Coulombic efficiency, and chemical oxygen demand (COD) removal and the effect of substrate concentration on electricity generation was determined. Microbial diversity of the anodic biofilms with different polyalcohols as carbon sources was also analyzed using denaturing gradient gel electrophoresis (DGGE).

2.2.1 Materials and methods

2.2.1.1 Chemicals

Xylitol [$C_5H_{12}O_5$, (2R,3R,4S)-Pentane-1,2,3,4,5-pentanol] and galactitol [$C_6H_{14}O_6$, (2R,3S,4S,5R)-hexane-1,2,3,4,5,6-hexol] were purchased from Aldrich (Milwaukee, WI, USA). Arabitol [$C_5H_{12}O_5$, (2R,4R)-pentane-1,2,3,4,5-pentol] was from Nutritional Biochemicals Corp. (Cleveland, OH, USA). Ribitol was from Pfanstiehl Laboratory (Waukegan, IL, USA). Mannitol [$C_6H_8(OH)_6$, (2R,3R,4R,5R)-hexane-

1,2,3,4,5,6-hexol] was from Matheson Coleman (Cincinnati, OH, USA), and sorbitol [$C_6H_{14}O_6$, (2R,3S,4S,5S)-hexane-1,2,3,4,5,6-hexol] was from Sigma Chemical Co. (St. Louis, MO, USA). All the other compounds were of analytical grade and obtained from commercial sources.

2.2.1.2 Inoculation and operation of microbial fuel cells

A medium solution (without carbon sources) was prepared by dissolving the following compounds in as reported and explained, previously (Lovley and Phillip, 1988). An acetate medium solution and six polyalcohol medium solutions were prepared by dissolving sodium acetate and each polyalcohol in the carbon free media solution.

The acetate culture medium solution (2000 mg L^{-1}, 7.0 mL) was added into each of the six MFCs, followed by a suspension of the electrochemically active bacteria (5 mL) that had been obtained from a MFC used in our previous study (Catal *et al.*, 2008a). Immediately following the addition of the bacteria suspension, MFCs were hooked up to a data acquisition system to start monitoring the voltage generation. When the voltage decreased below 50 mV, sodium acetate medium solution in each MFC was refreshed. When a stable power output was obtained at an external resistance of 1k Ω, the sodium acetate solution was replaced with each polyalcohol medium solution with the following concentrations: 8 mM (1220 mg L^{-1}) for xylitol, arabitol and ribitol, and 6.7 mM (1220 mg L^{-1}) for mannitol, galactitol and sorbitol. Difference in the molar concentration of the polyalcohols was to standardize the total organic carbon concentration (480 mg L^{-1}) in solution.

The external resistance was varied from 1k to 50 Ω in order to prepare the polarization curves. At each resistance, MFCs were operated for at least two batches to ensure repeatable voltage output. Concentrations of polyalcohols were varied in the range of 150-2400 mg L^{-1} to investigate their effect on electricity production at a fixed resistance of 120 Ω. The six MFCs with each containing a different polyalcohol were run simultaneously in a constant temperature chamber (32 °C). All the experiments were replicated twice. Analyses and calculations were performed as described in section 2.1.2.3.

2.3 Effects of Furan Derivatives and Phenolic Compounds on Electricity Generation

MFCs are devices that directly covert chemical energy to electricity through catalytic activities of microorganisms. One of the greatest advantages of MFCs over hydrogen- and methanol-fuel cells is that a diverse range of organic materials can be used as fuels (Logan and Regan, 2006; Lovley, 2006a). Electricity has been generated in MFCs from various organic compounds, including carbohydrates (Liu and Logan, 2004; Rabaey *et al.,* 2005; Catal *et al.,* 2008a), proteins (Heilmann and Logan, 2006) and fatty acids (Liu *et al.,* 2005; Cheng *et al.,* 2006). Lignocellulosic biomass is an attractive fuel source for MFCs due to its renewable nature and ready availability. Our recent study demonstrated that all monosaccharides that can be directly generated from hydrolysis of lignocellulosic biomass were good sources for electricity generation in MFCs (Catal *et al.,* 2008a). However, lignocellulosic biomass cannot be directly utilized by microorganisms in MFCs for electricity generation. In other words, lignocellulosic biomass has to be converted to monosaccharides or other low-molecular-weight compounds (Ren *et al.,* 2007). The most commonly used method of converting lignocellulosic biomass to monosaccharides is through a dilute-acid pre-treatment and subsequent acid- or enzymatic hydrolysis processes (Taherzadeh *et al.,* 1999). In addition to monosaccharides, the dilute-acid pretreatment and the subsequent acid hydrolysis generate a number of byproducts, such as furan derivatives (2-furaldehyde and 5-hydroxymethyl-2-furaldehyde), phenolic compounds and carboxylic acids (acetic, formic, and levulinic acids) (Almeida *et al.,* 2007; Cantarella *et al.,* 2004). These byproducts negatively affect the cell membrane function, growth, and glycolysis in ethanol-producing yeast and bacteria (Taherzadeh *et al.,* 1999; Larsson *et al.,* 2001; Clark and Mackie, 1984; McMillan, 1994; Klinke *et al.,* 2002). However, some of these byproducts such as acetic acid are good substrates for electricity-generating microbes (Liu *et al.,* 2005; Lovley and Phillips, 1988). A hydrolysate from a dilute-acid pretreatment of corn stover could even be directly used for electricity generation in MFCs (Zuo *et al.,* 2006). However, the effects of these individual byproducts on electricity generation in a MFC are still poorly understood.

In this part of the study, ten selected compounds that are either known byproducts in an acid-pretreatment or acid-hydrolysis of lignocellulosic biomass or model compounds of those byproducts were thoroughly investigated as substrates in a MFC for electricity generation.

2.3.1 Testing of the compounds and experimental design

2.3.1.1 Materials

The following chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI, USA) and used as received: 3,5-dimethoxy-4-hydroxycinnamic acid, 4-hydroxycinnamic acid, syringaldeyhde, *trans*-4-hydroxy-3-methoxycinnamic acid, and 3,4-dimethoxybenzyl alcohol. Acetophenone, 2-furaldehyde, and 5-(hydroxymethyl) furfural were purchased from Acros Organics (Morris Plains, NJ, USA). *Trans*-cinnamic acid was obtained from Eastman (Kingsport, TN, USA) and vanillin was from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals such as glucose and sodium phosphate were purchased from commercial sources. Non-wet proofing carbon cloth (type A) and wet-proofed (30%) carbon cloth (type B) were purchased from E-TEK (Somerset, NJ, USA) and used as electrodes in MFCs. A multimeter (model 2700) with a data acquisition system (Keithly Instruments Inc., Cleveland, OH, USA) was used for measuring voltage in a MFC. Electrically active bacteria that had been enriched from wastewater in Corvallis Wastewater Treatment Plant (Corvallis, OR) and used in our previous study were also used in this study (Catal *et al.,* 2008a). MFCs were constructed as described in the section of 2.1.2.1.

2.3.1.2 Microbial fuel cell operation with selected compounds used as sole carbon sources for electricity generation

A vitamin stock solution and a mineral stock solution were prepared according to literature procedures, and the same as used in the previous experiment (Lovley and Phillips, 1988). A glucose-free culture media solution was prepared by dissolving the following compounds in water at room temperature as described previously. A glucose-containing culture media was prepared separately by adding glucose (1200 mg L^{-1}) into the glucose-free culture media solution. The stock solutions of the individual furan derivatives and phenolic compounds were prepared by adding each

compound in the following amount to the glucose-free culture medium (50 mL) (40 mM): 5-HMF (64 mg), syringaldehyde (45.5 mg), vanillin (38 mg), *trans*-cinnamic acid (37 mg), *trans*-4-hydroxy-3-methoxy-cinnamic acid (48.5 mg), 4-hydroxy-cinnamic acid (41 mg), 3,5-dimethoxy-4-hydroxy-cinnamic acids (56 mg). These stock solutions were diluted with the glucose-free medium solution 1 mL stock solution + 39 mL glucose-free medium to obtain 1 mM concentration of the compound and used to investigate voltage generation from these compounds in the absence of other carbon sources.

The glucose-containing culture medium solution (7.0 mL) was added into each of the MFCs, followed by a suspension of the electrically active bacteria (5 mL) that had been obtained and used for our previous study (Catal *et al.,* 2008a). Immediately after adding the bacteria suspension, MFCs were attached to a data acquisition system to start monitoring the voltage generation. When a stable voltage output was obtained in the MFCs, the glucose-containing culture media solution was replaced by the furan derivatives and phenolic compounds solutions. Ten MFCs were operated in a batch-fed mode simultaneously.

2.3.1.3 Microbial fuel cell operation to study the effects of selected compounds on electricity generation from glucose

The stock solutions of furan derivatives and phenolic compounds prepared in *section 2.3* were diluted with glucose-free medium solution to obtain final concentrations ranged from 0.01 to 40 mM. Glucose was added to each of the diluted solutions to obtain a 1200 mg L^{-1} final concentration.

MFCs used in this set of experiments were started up following the same procedures as described in *section 2.1*. When a stable voltage output was obtained, the glucose-containing medium solution was replaced by a medium solution containing both glucose and one of the furan derivations or phenolic compounds (0.01 mM). At the end of the batch (voltage output less than 50 mV), the solution was replaced with fresh medium solution containing a higher concentration of the selected compound. The concentration of the selected compound was continuously increased until a significantly reduced voltage generation was observed. The medium solution was

then replaced with the glucose-containing (without selected compound) medium solution to investigate if voltage generation could be recovered.

All MFCs were operated at a fixed external resistance of 1k Ω and kept in an incubator with a constant temperature of 30±2 °C throughout the experiments.

2.4 Substrate Mixture Utilization in Microbial Fuel Cells

The MFC technology needs to be improved in order to become economically feasible, and in that respect lignocellulosic biomass which contains high carbohydrate stock may help decrease the process costs (Logan *et al.,* 2006; Catal *et al.,* 2008a). The scale-up of this technology also involves dealing with some problems such as isolation of super-electricity generating microorganisms (Liu *et al.,* 2008) obtaining higher power output and determination of substrate utilization patterns for process optimization. The operation for electricity production using lignocellulosic biomass requires pretreatment methods and produces complex sugar mixtures of which the utilization patterns in MFCs are still unknown.

Lignocellulosic materials have to be degraded smaller molecules before they can be utilized by the microorganisms in a MFC for generation of electricity or hydrogen gas. Woody biomass mainly consists of five monosaccharides: D-xylose, D-glucose, D-mannose, D-galactose, and L-arabinose. These monosaccharides have different chemical structures and their metabolic pathways by the microorganisms in a MFC for electricity generation are expected to be different. Hydrolysis of woody biomass typically results in a mixture of monosaccharides. Here, we investigated whether microorganisms in a MFC preferentially used each individual monosaccharide for electricity generation.

2.4.1 Techniques and analytical methods

2.4.1.1 Microbial fuel cell operation

All experiments were conducted in a constant temperature room (32 °C), and 1k ohm resistance was initially used. Mixed microorganism culture was obtained from Corvallis Wastewater plant (OR, USA), inoculated into MFCs (using 5 mL) and

microorganisms were enriched using glucose (1200 mg L^{-1}) before experiments. The medium composition was prepared as described, previously in section 2.1.2.2 (Lovley, 1988). The solution was replaced with one the following solutions containing different sugars: (1) glucose and galactose, (2) galactose and mannose, (3) glucose and xylose, (4) arabinose and xylose (500 mg L^{-1} for each sugars), (5) glucose, galactose, mannose, arabinose, and xylose (500 mg L^{-1} for each one) in combination. Effluent samples were collected during the operation in every 10 mins.

2.4.1.2 Analytical Techniques

Samples for sugar determinations were collected from the MFCs, and filtered through 0.2 μm pore-diameter cellulose acetate syringe filter (Millipore Corp.) immediately before analysis. Samples were then analyzed using high-pressure liquid chromatography (HPLC, Waters) using a refractive index detector and an Aminex HPX-87P (300 mm x 7.8 mm) column (Biorad Laboratories, Hercules, CA, USA) at 56 °C. Deionized water was used as the mobile phase at a flow rate of 0.6 mL min^{-1}, and helium gas was purged to mobile phase for 15 min before the analysis. Injection volume was 20 μL. A software was used to calculate the sugar amounts (Millenium).

Volatile fatty acids (VFAs) in the medium were analyzed using a gas chromatography (GC) (Agilent Technologies, 6890N Network GC System, Serial no. CN61439161, China) equipped with an injector (Agilent Technologies, 7683B Series) adding phosphoric acid. VFAs standard mixture (diluted in deionized water, Supelco, Bellefonte, PA, USA) was used as standard. All GC analysis was monitored using a software (ChemStation for GC Systems, 6890).

2.5 Microbial Fuel Cell Operational Factors

MFCs can also be used for wastewater treatment which may provide inexpensive carbon substrates (Liu *et al.,* 2004). MFCs have been developed in order to eliminate the disadvantages of inorganic fuel cells such as high cost of catalysts, high operation temperature, and requirement of extreme corrosives (Liu and Logan, 2004). Unlike inorganic fuel cells, MFCs can be operated under mild reaction conditions, namely ambient operational temperature and pressure. However, the process of MFCs needs to be evaluated in some aspects including several factors such as the effect of pH and

substrate concentration on electricity generation. The effects of pH on electricity production from wastewater in MFCs have not yet researched well.

2.5.1 Effect of pH

Evaluation of pH effect in MFC is very important and still under investigation. Previously, Gil *et al.* (2002) studied the operational parameters including pH which affects the performance of mediator-less microbial fuel cells using wastewater (Gil *et al.,* 2002). Bioreactor based electricity production has been suggested as a novel approach for wastewater treatment (Rabaey *et al.,* 2006). Rabaey *et al.,* (2005) have reported a novel MFC where anode part of the cell consists of granular graphite that permit wastewater to flow through the system and serve as surface area for bacteria to form biofilm structure. He *et al.,* (2006) have reported another kind of MFC in a tubular design which wastewater flows from the bottom to the top feeding continuously with artificial wastewater containing sucrose solution (He *et al.,* 2006). Neverthless, the reported power output using MFC was not yet high enough for scale-up. Recently, single chamber MFCs have been introduced (Liu and Logan, 2004), and here a single-mediator-less MFC was used which does not require exogenous chemicals to provide electron transfer to the electrode. Operation is easy and can be affected by various paramateres such as temperature, and pH (Oh *et al.,* 2004).

2.5.2 Microbial community

2.5.2.1 DNA Extraction and Polymerase Chain Reaction (PCR) amplification

Biofilms were scratched from the anodes of MFCs run in the presence of different monosaccharides and polyalcohols for 20 days (around 10 batches). Bacterial genomic DNA was extracted from the biofilm samples using DNeasy tissue Kits (Qiagen, CA, USA) according to the manufacturer's instructions. The universal primer set 357F-GC (5'-GC-clamp-CCTACGGGAGGCAGCAG-3') and 518R (5'-ATTACCGCGGCTGCTGG-3') (Invitrogen, Carlsbad, CA, USA) was used to amplify the V3 region of bacteria 16S ribosomal DNA (rDNA) from the extracted genomic DNA (Muyzer *et al.,* 1993). PCR amplification was performed in a thermocycler (Thermo hybaid, MBS 0.2G, Thermo, MA, USA). PCR mixture (per

25 µl of reaction mixture) contained 12.5 µl GoTaq Green Master Mix (Promega, Madison, WI, USA), 0.5 µM of each of the primers and 100 µg of template. PCR cycling was carried out under the following conditions: an initial denaturation at 94°C for 3 min followed by 30 cycles consisting of denaturation at 94 °C for 30 s, primer annealing at 54 °C for 30 s and extension at 72 °C for 30 s. A final extension step was conducted at 72 °C for 10 min prior to cooling at 4 °C (Muyzer *et al*., 1993; Catal *et al.*, 2008c).

2.5.2.2 Denaturing gradient gel electrophoresis (DGGE) method

DGGE of the PCR products was carried out in a Dcode^{TM} Universal Mutation Detection System (Bio-rad Laboratories, Hercules, CA, USA). The 8% (w/v) polyacrylamide gels (16 cm×16 cm gel, thickness of 1 mm) contains 30% to 55% denaturing gradients (urea and formamide). Electrophoresis was conducted using a 1×TAE (Tris-Acetate-EDTA) buffer at 130V and 60 °C for 5 hours. After the electrophoresis, the gel was stained with 1µg/mL ethidium bromide (American Bioanalytical, Natick, MA, USA) in 1×TAE buffer for 15 min and detained in 1×TAE buffer for 10 min. The fragments were visualized under a UV transilluminator (Muyzer *et al*., 1993; Catal *et al.*, 2008c).

2.6 Evaluation of Pine Wood Flour Acidic Hydrolysate in Microbial Fuel Cells

Utilization of lignocellulosic materials for production of any kind of energy is very popular subject in recent years due to its high carbohydrate content (Petrus and Noordermeer, 2006). Ethanol, hydrogen, and electricity generation from lignocellulosic materials were previously published by many researchers, and the most challenging assignment is to decrease the process costs and increasing the energy yield to maximize utilization of lignocellulosic materials by microorgamisms in bioprocesses. Pine wood flour constitutes one the largest bodies of woody biomass waste, and is produced by agricultural and wood industries. As other woody biomass resources, pine wood flour also consists of hemicellulose, cellulose, and lignin. Utilization of this kind of products generally requires pretreatment methods such as steam treatment, or hydrolysis (acidic/enzymatic) to make sugar content of lignocellulosic materials available for microorganisms (Klinke *et al.,* 2002).

MFCs are biotechnological instruments to produce an energy form, electricity, using carbon sources by microorganisms (Logan and Regan, 2006). Electricity generating bacteria oxidize organic substances in the chamber, and part of the removed electrons coming from organic materials are transferred through an external circuit from anode to cathode, producing water in air cathode single chamber MFCs (Liu *et al.,* 2004). Bacteria which were reported producing electricity can utilize a wide range of substrates such as carbohydrates, proteins, small peptides. Rezaie *et al.*,(2007) have reported electricity generation by complex carbohydrates such as cellulose, and chitin. Catal *et al.* (2008a) also reported wide range of monosaccharides as potential substrates for direct electricity generation. Phenolic compounds which are formed during the hydrolysis of lignocellulose such as cinnamic acids, vanillin, syringaldehyde, and 5-HMF were not observed as inhibitors in MFCs (Catal *et al.,* 2008b). Zuo *et al.* (2006) have previously reported electricity generation from another lignocellulosic matter, corn stover, applying dilute acidic treatment to produce sugars. Various polyalcohols have been suggested as substrates for electricity generation (Catal *et al*., 2008c). However, this treatment step is one the major challenges in the potential utilization of lignocellulosic material to produce electricity because of the process costs.

In this part of the study, we examined the electricity generation directly from pine wood flour under hydrolytic conditions.

3. RESULTS AND DISCUSSION

3.1 Studies of Electricity Production Using Monosaccharides

3.1.1 Voltage output and adaptation time

Sodium acetate was used as the carbon source for all 12 MFCs during the start-up period. When a stable power was generated, culture medium was replaced with a monosaccharide solution. All monosaccharides produced electricity without the addition of new bacterial inoculum (Fig. 3.1-12). However, the adaptation time, which was defined as the time between adding a monosaccharide solution to a MFC and reaching a maximum power output at 1k Ω, varied for different monosaccharides. The bacteria easily adapted to glucose, and, the adaptation time was very short (less than 1 h) (Fig. 3.1). Longer adaptation time was required for gluconic acid (ca. 7 h) compared to glucose (Fig. 3.12). While the adaptation time was similar (around 12-18 h) for fructose, galactose, fucose, mannose, xylose, galacturonic acid and glucuronic acid under the same conditions (Fig. 3.1-12), it was much longer for arabinose (ca. 60-70 h) (Fig 3.8). Once the bacteria adapted to a new monosaccharide, electricity was quickly recovered when the monosaccharide solution was refreshed.

Pure cultures of various electricity-generating bacteria can utilize certain substrates only. For example, the carbon source that *Geobacter* species could use was primarily limited to simple organic acids such as acetate (Chaudhuri and Lovley, 2006; Bond and Lovley, 2003). *Pseudomonas* species isolated from the MFC with glucose as carbon source could not further utilize the fermentative products, such as acetate, for electricity generation (Rabaey and Verstraete, 2005; Rabaey *et al.,* 2004). *Shewanella* species could only incompletely oxidize a limited number of organic acids such as lactate and pyruvate to acetate under anaerobic conditions (Bond and Lovley, 2003; Rabaey *et al.,* 2004), limiting the efficiency of electricity production.

Results from this and previous studies appear to suggest that a mixed bacterial culture is superior to a pure bacterial culture in terms of electricity generation, especially when a mixture of carbon sources are used (Rabaey and Verstraete, 2005 ; Liu and Logan, 2004; Zuo *et al.*, 2006; Min *et al.*, 2005).

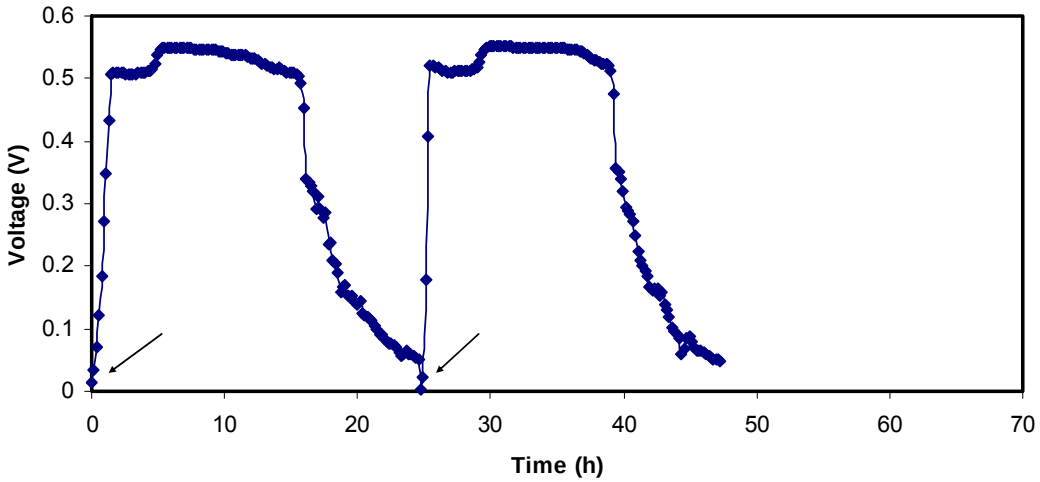

Figure 3.1: Voltage generation from glucose at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

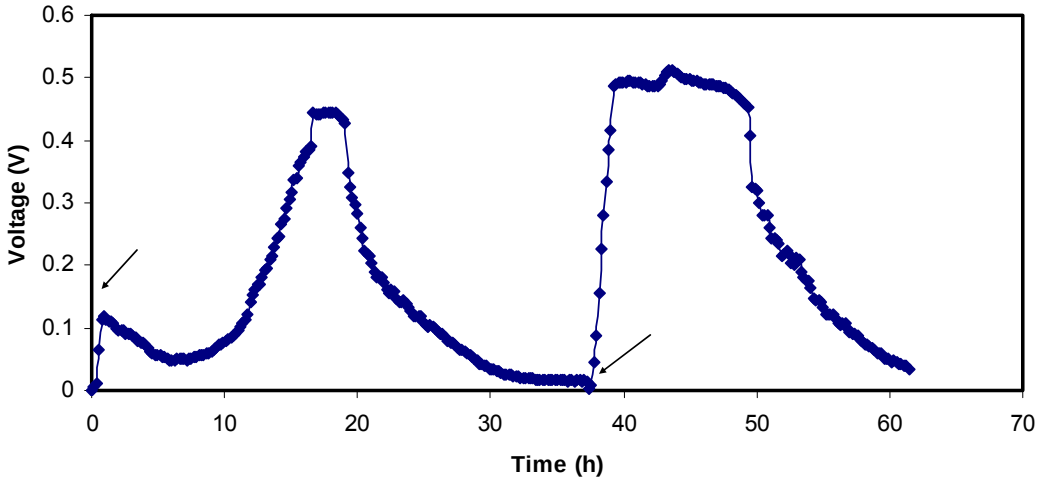

Figure 3.2: Voltage generation from mannose at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

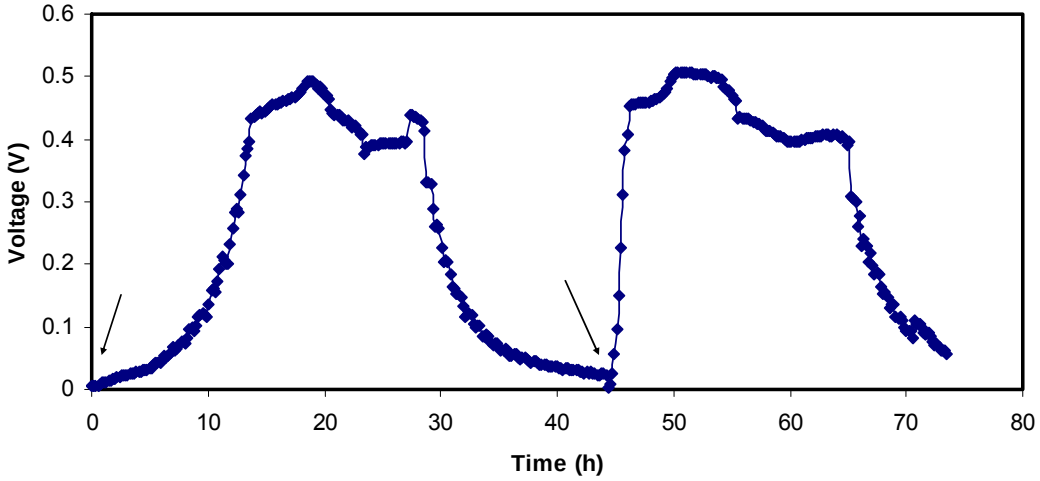

Figure 3.3: Voltage generation from rhamnose at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

Galactose produced electricity in MFCs. Figure 3.4 shows voltage generation from galactose.

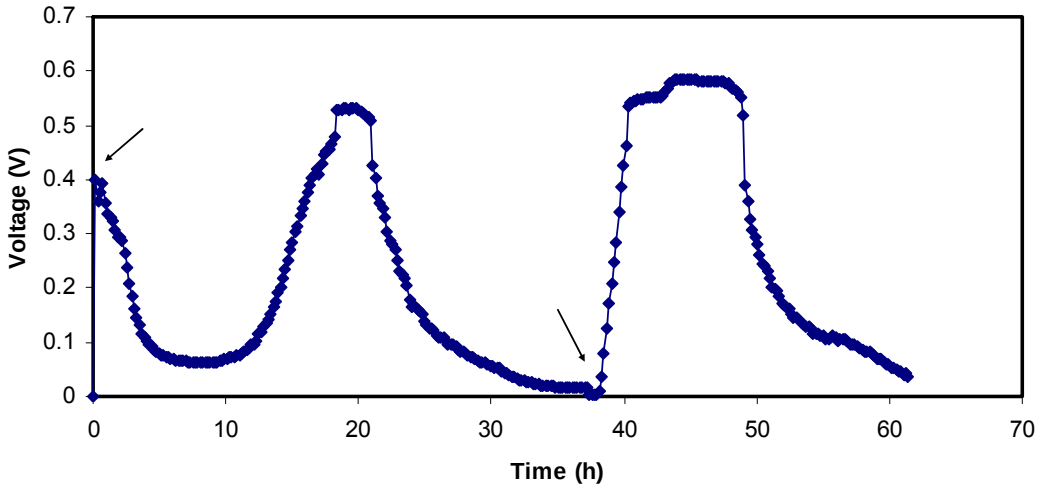

Figure 3.4: Voltage generation from galactose at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

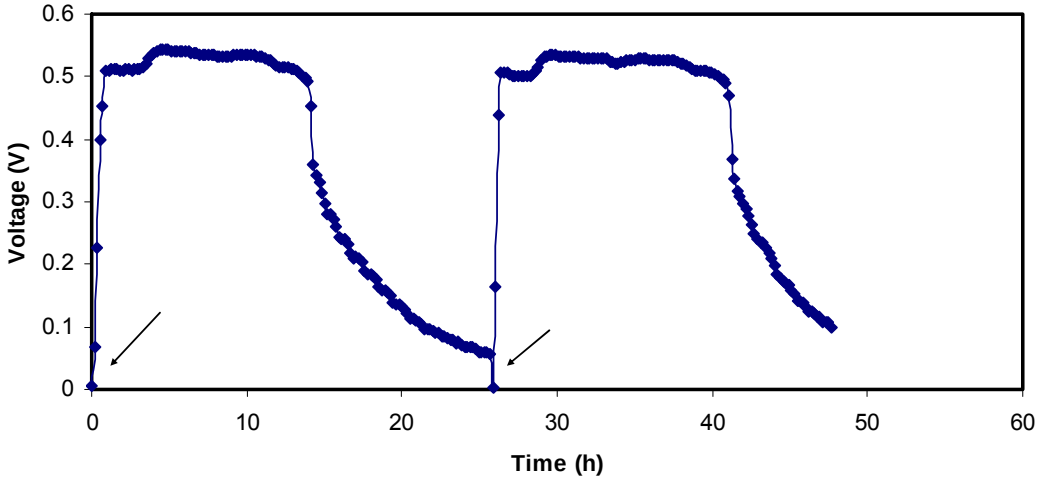

Figure 3.5: Voltage generation from fructose at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

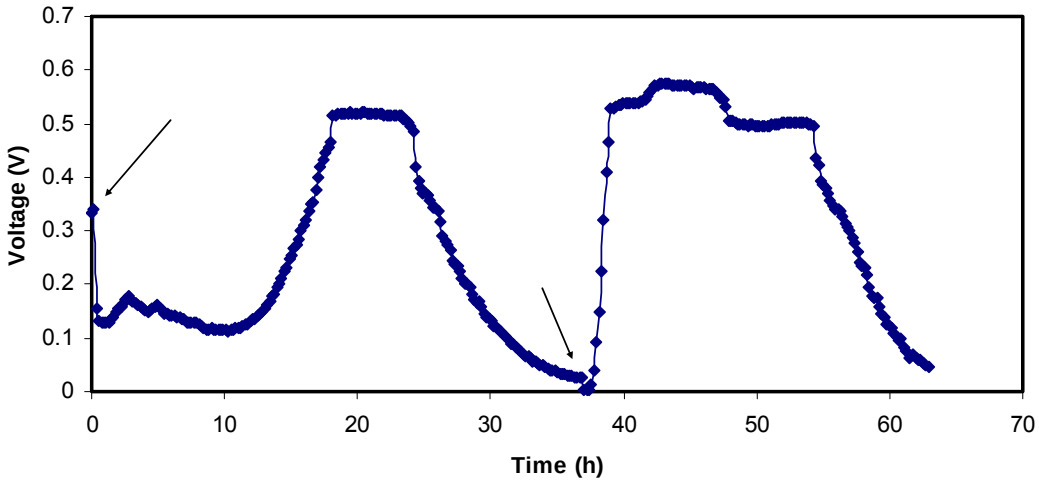

Figure 3.6: Voltage generation from fucose at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

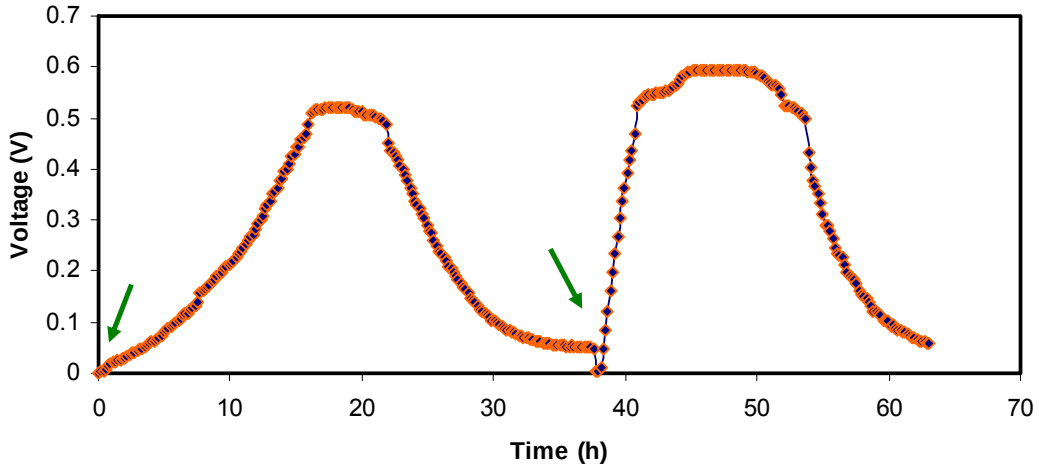

Figure 3.7: Voltage generation from pentose sugar, xylose at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

Arabinose produced electricity, and the adaptation time was about 70 h.

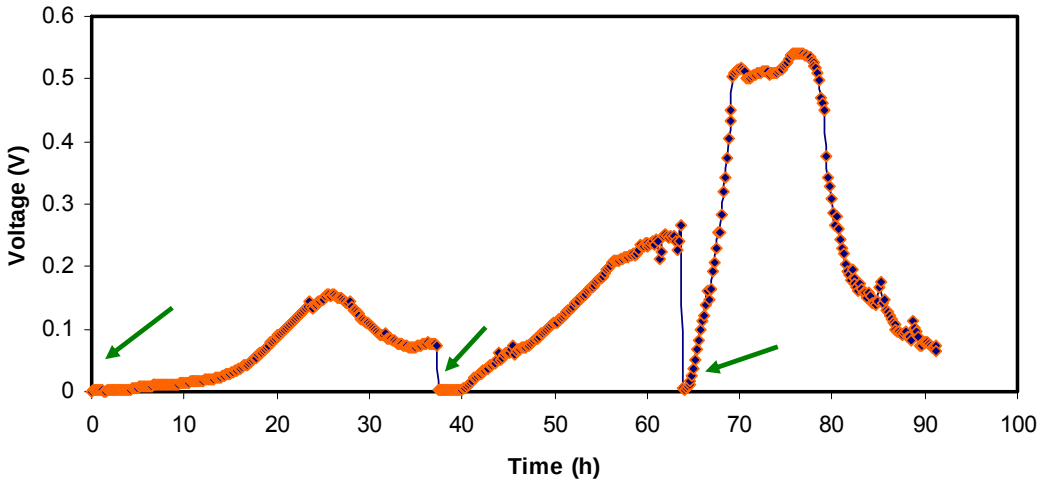

Figure 3.8: Voltage generation from pentose sugar, arabinose at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

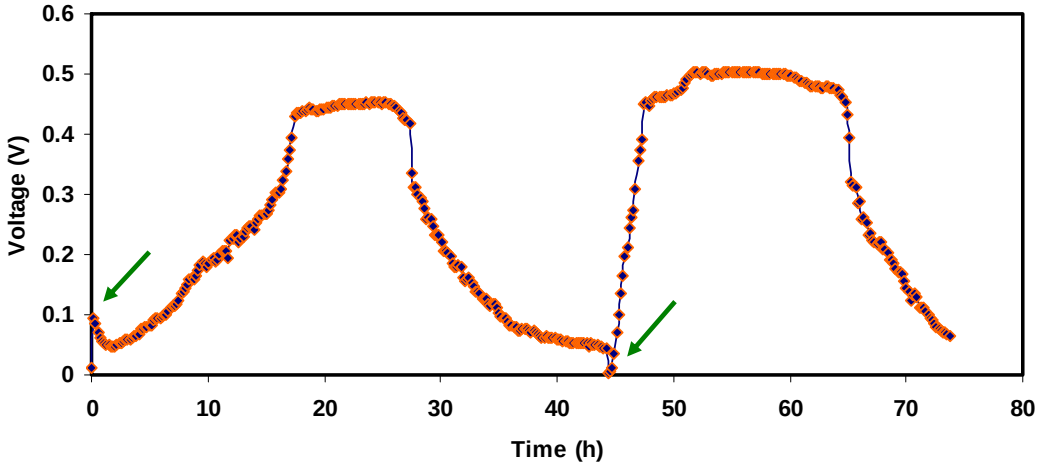

Figure 3.9: Voltage generation from pentose sugar, ribose at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

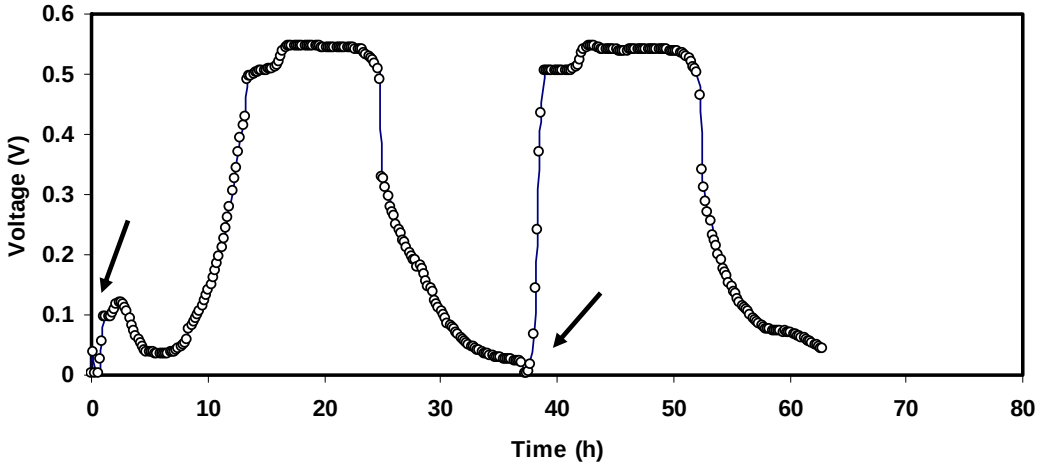

Figure 3.10: Voltage generation from sugar derivative, galacturonic acid at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

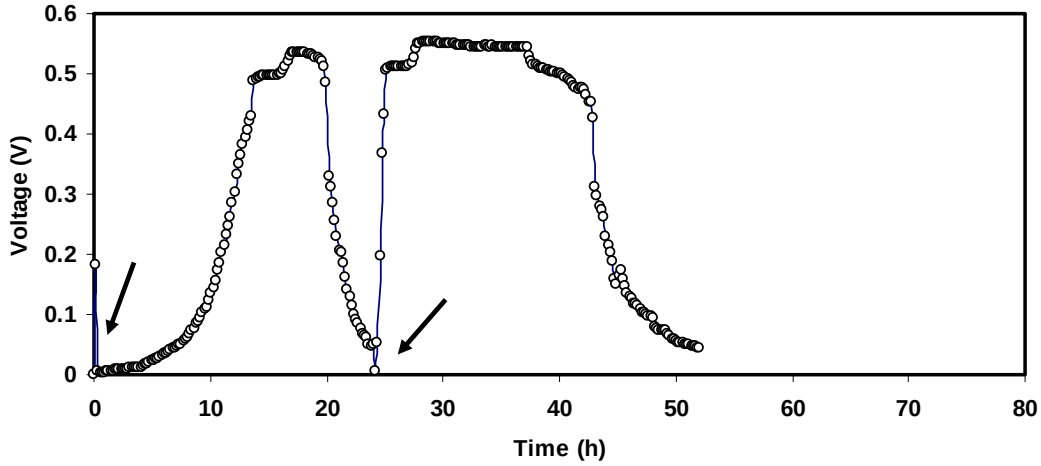

Figure 3.11: Voltage generation from sugar derivative, glucuronic acid at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

Electricity was produced by gluconic acid (Figure 3.12).

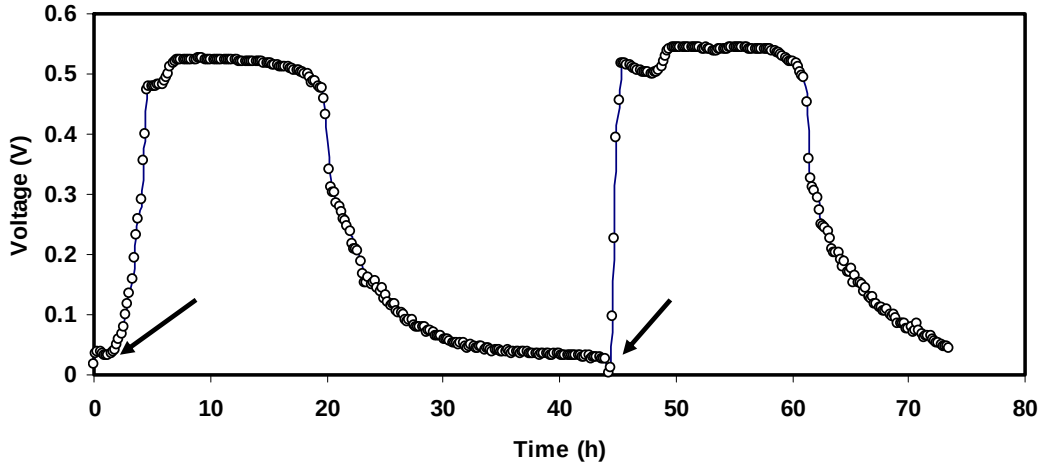

Figure 3.12: Voltage generation from sugar derivative, gluconic acid at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

3.1.2 Power densities obtained in the presence of monosaccharides

The maximum power density of MFCs with each monosaccharide was determined by varying the circuit resistance from 1k to 50 Ω. For hexoses, glucose resulted in the highest maximum power density of 2164 mW m^{-2} at a current density of 0.7 mA

cm^{-2} , whereas mannose had the lowest one, 1407 mW m^{-2} (Fig. 3.13A). The maximum power densities for galactose, fructose, fucose and rhamnose were 2094 mW m^{-2}, 1775 mW m^{-2}, 1773 mW m^{-2} and 1430 mW m^{-2}, respectively (Fig. 3.13A).

Xylose and arabinose are major pentoses in lignocellulosic material while ribose content is fairly low (Bjerre *et al.,* 1996; Pan and Sano, 2005). Xylose generated the maximum power density of 2331 mW m^{-2} at a current density of 0.74 mA cm^{-2}, which was higher than those from arabinose (2031 mW m^{-2}) and ribose (1518 mW m^{-2}) (Fig. 3.13B). As a matter of fact, the maximum power density from xylose was even higher than that from glucose. Xylose was reported as one of the major constituent of corn-stover acid hydrolysates (32.88 g L^{-1}) along with glucose (9.83 g L^{-1}) (Zuo *et al.,* 2006).

Glucuronic acid resulted in a maximum power density of 2801 mW m^{-2} at a current density of 1.18 mA cm^{-2}, which was 35% higher than that with gluconic acid and 86% higher than that with galacturonic acid (Fig. 3.13C). The maximum power density from glucuronic acid was even higher than those provided by glucose and xylose, indicating that glucuronic acid was a good substrate for electricity generation.

3.1.3 Effect of monosaccharide concentration on voltage generation

For all monosaccharides tested, the maximum voltage output at 120 Ω external resistance initially increased with the monosaccharide concentration, however, further increases above a certain level did not improve the electricity generation (Fig. 3.14). The maximum voltage ranged from 0.26 V to 0.44 V and a half-saturation constant (Ks) ranged from 110 mg L^{-1} to 725 mg L^{-1} (R^2 =0.826 – 0.995) (Table 3.1). Glucose produced the highest maximum voltage (0.39 V) with Ks=637 mg L^{-1} (R^2 =0.993) and rhamnose produced the lowest voltage (0.27 V) with Ks= 283 mg L^{-1} (R^2 =0.826) among the six hexoses tested. Xylose resulted in a higher maximum voltage (0.38 V) than two other pentoses (arabinose and ribose). Although similar half-saturation constants were obtained with xylose and arabinose, a higher power output was achieved with xylose. Glucuronic acid provided a higher maximum voltage (0.44 V) with Ks=725 mg L^{-1} (R^2 =0.987) than did gluconic acid and galacturonic acid (Table 3.1). The predicted half-saturation constant of gluconic acid was higher than that of galacturonic acid, and a higher maximum power output was

obtained with glucuronic acid. The half-saturation constant of glucose in this study was about 5.2 times higher than that from the previous report using the same substrate (Liu and Logan, 2004). The difference was mainly due to the selection of different resistances in these studies. When an MFC is operated at a high external resistance, the electron transfer rate from bacteria to anode could be limited by the external resistance and increasing the substrate concentration will not increase the power output. The reduction of the external resistance from 1k to 120 Ω allowed us to better evaluate the effect of the monosaccharide concentration on the voltage output of the MFCs tested since the maximum power outputs occurred at a resistance range of 110 to 220 Ω for most of the monosaccharides tested in this study.

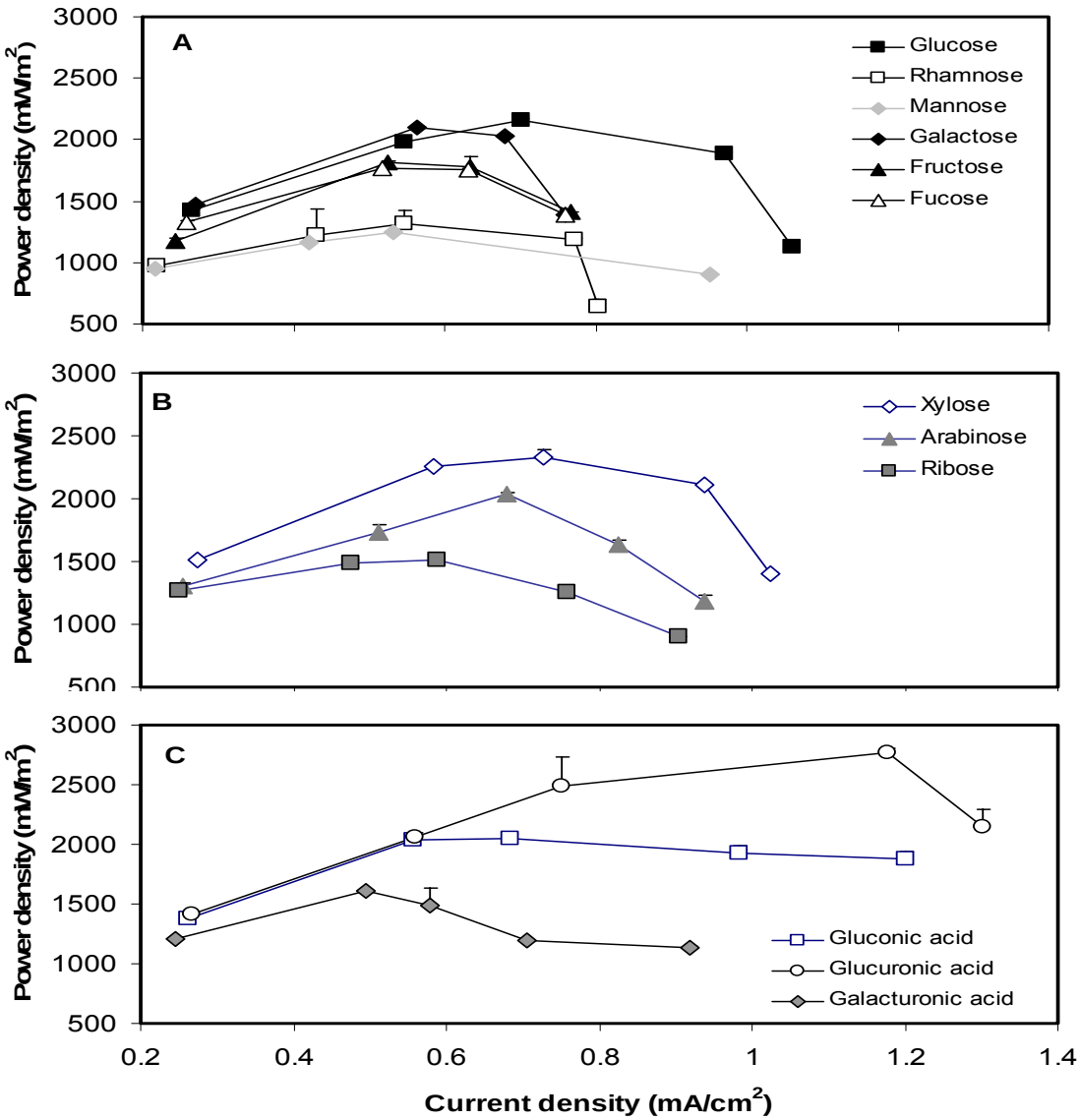

Figure 3.13: Power density as a function of current density obtained from monosaccharides.

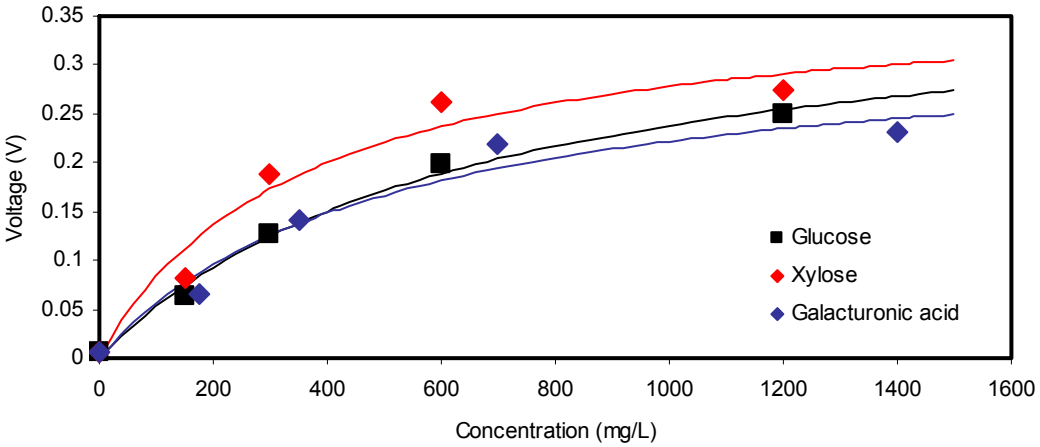

Figure 3.14: Effects of monosaccharide concentrations on voltage output.

3.1.4 Chemical oxygen demand removal and coulombic efficiency

For all monosaccharides tested, about 80-95% of COD was removed at the end of the experiment when the voltage was lower than 0.05 V (Table 3.1). However, the E_c that was calculated based on the total substrate concentration was only in the range of 21 to 37% at 120 Ω, indicating that a substantial amount of electrons were lost. Many factors could contribute to the electron loss in the air-cathode MFCs, including the electron transfer from substrate to other electron acceptors in solution, such as nitrate, sulfate, and oxygen, and the substrate utilization for bacterial growth, fermentation, and/or methanogenesis (Liu and Logan, 2004). While the nitrate and sulfate concentrations were very low (< 0.35 mmol L^{-1}) in the solution, significant amount of oxygen could be diffused through the membrane-free air cathode and oxidize the substrate by aerobic bacteria (Liu and Logan, 2004). A modification of the single chamber air cathode by adding a cloth layer on to the cathode surface significantly reduced the oxygen diffusion and enhanced the E_c in a recent study (Fan *et al.*, 2007). Although the biomass yield on anode was generally low for anaerobic bacteria compared to aerobic bacteria (Rabaey and Verstraete, 2005), the aerobic bacterial growth on the cathode surface (Liu and Logan, 2004) and the anaerobic/aerobic bacteria in the solution may account for a significant decrease in the E_c. Using monosaccharides, the bacterial concentrations in solution at the end of the batch were much higher (1.5-4.5 fold) than that using acetate (OD_{600nm}=0.069) , and arabinose was the only exception (0.069) (Table 3.1). Compared to that of

acetate (59 %), faster utilization rates of these monosaccharides in bacterial growth might be attributed to the lower E_c of MFCs.

Table 3.1: MFC performance with monosaccharides.

Carbon sources		Power density (mW/m^2)	E_c[a] (%)	COD removal (%)	OD_{600nm}[b]	V_{max} (V)	Ks ($mg\ L^{-1}$)	R^2 (%)
Hexose	Glucose	2164±2	28	93±2	0.245	0.39	637	0.993
	Galactose	2094±3	23	93±2	0.289	0.35	403	0.960
	Fructose	1814±9	23	88±2.3	0.180	0.31	275	0.985
	Fucose	1757±11	34	84±4.0	0.156	0.35	383	0.995
	Rhamnose	1318±110	30	90±1.6	0.127	0.27	283	0.826
	Mannose	1243±5	25	88±3.5	0.178	0.29	322	0.974
Pentose	Xylose	2331±62	31	95±2.1	0.131	0.38	352	0.960
	Arabinose	2031±20	27	93±2.3	0.069	0.26	111	0.996
	Ribose	1518±4	30	86±3.1	0.103	0.27	447	0.955
Sugar derivates	Galacturonic	1611±6	22	80±1.7	0.169	0.33	493	0.964
	Glucuronic	2767±27	24	89±0.8	0.207	0.44	725	0.987
	Gluconic acid	2054±27	30	93±5.6	0.313	0.28	580	0.963

[a] Coulombic efficiencies at 120 Ω external resistance

[b] Optical density at 600 nm of the MFC solution sampled at the end of the initial batch

3.2 Electricity Generation Using Disaccharides as Substrates

3.2.1 Voltage output and adaptation time

Electricity was produced from all disaccharides tested, including D-maltose and D-cellobiose (Fig. 3.15-3.16). The mixed bacterial culture enriched using sodium acetate as a carbon source adapted well to all carbon sources tested. The adaptation

time, which was defined as the time between adding a disaccharide solution to a MFC and reaching a maximum power output at 1k Ω, was similar for each disaccharide. However, once the bacteria adapted to a new disaccharide, electricity was quickly recovered when the disaccharide solution was refreshed.

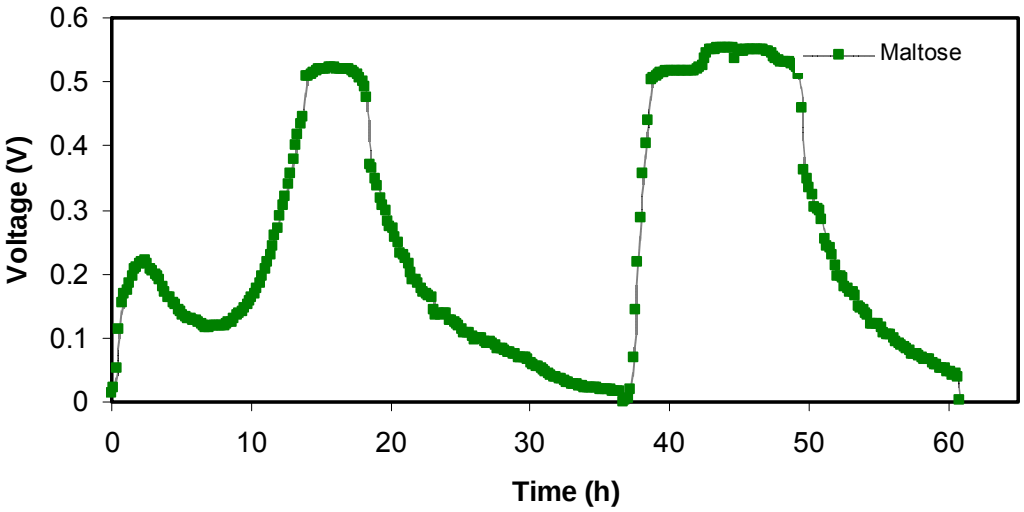

Figure 3.15: Voltage generation from maltose at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

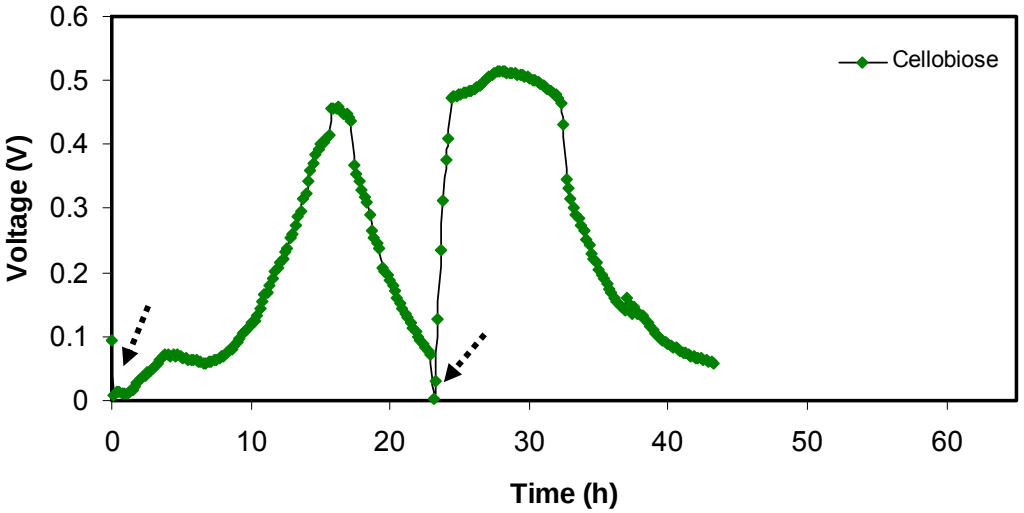

Figure 3.16: Voltage generation from cellobiose at 1 kΩ external resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

3.2.2 Power generation in the presence of disaccharides

Maximum power density obtained from the disaccharides were 1262±5 mW m^{-2} for D-cellobiose, 1893±67 mW m^{-2} for D-maltose at current density of 0.44 and 0.66 mA cm^{-2}, respectively (Fig. 3.17-3.18).

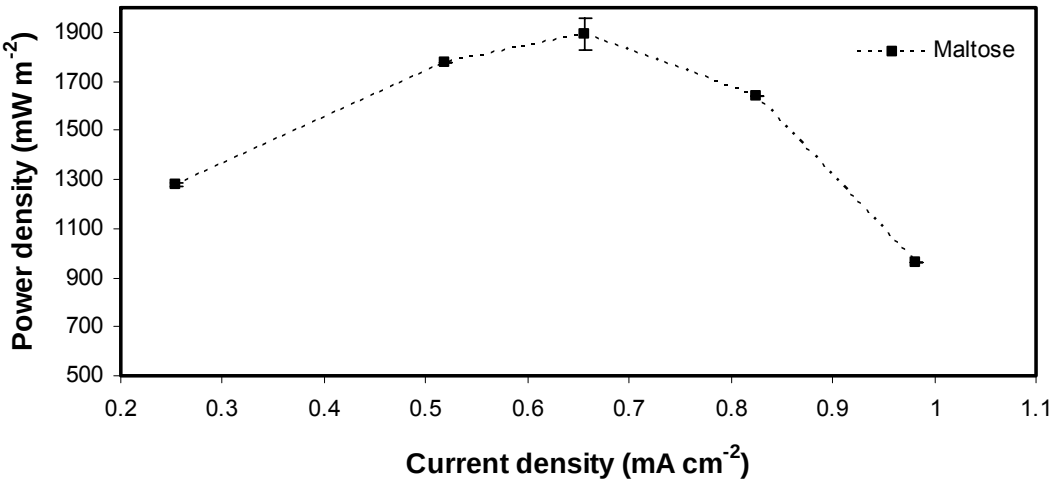

Figure 3.17: Power density as a function of current density obtained from maltose.

Figure 3.18 shows power density result obtained from cellobiose.

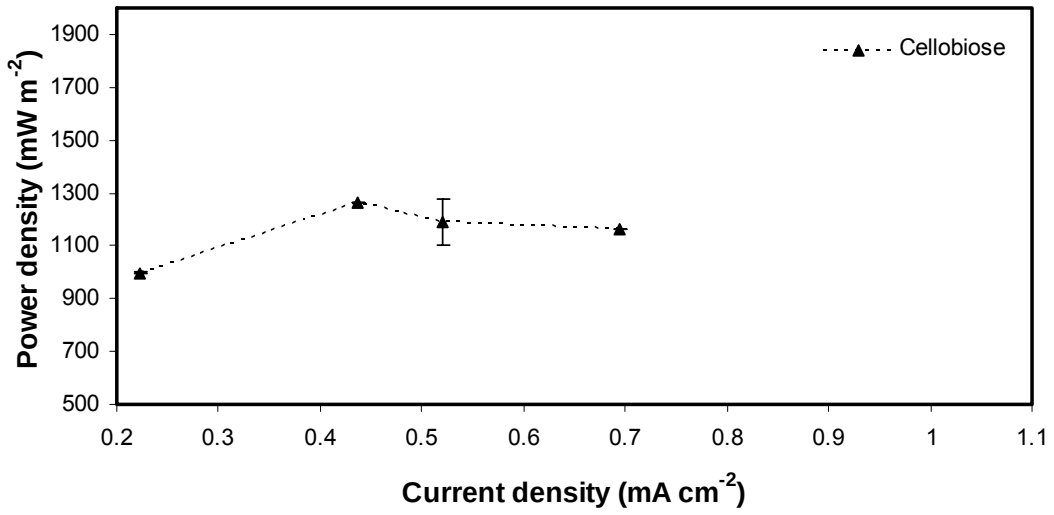

Figure 3.18: Power density as a function of current density obtained from cellobiose.

3.2.3 Effects of disaccharide concentrations on voltage and Coulombic efficiency

For the two disaccharides tested, the maximum voltage output at 120 Ω external resistance initially increased with disaccharide concentration; however, increases above a certain level did not improve the electricity generation (Fig. 3.19-3.20). Coulombic efficiency was 18% for D-cellobiose and 30% for D-maltose. The relationship between the maximum voltage output and the substrate concentration appeared to follow saturation kinetics at 120 Ω external resistance. The estimated maximum voltage output ranged between 0.34-0.40 V and half-saturation kinetic constants varied from 626 to 733 mg L^{-1} for D-cellobiose and D-maltose, respectively. Chemical oxygen demand (COD) removal was above 81 % for the disaccharides tested (Table 3.2). Results indicated that lignocellulosic biomass-derived disaccharides might be a suitable substrates for electricity generation using MFC technology.

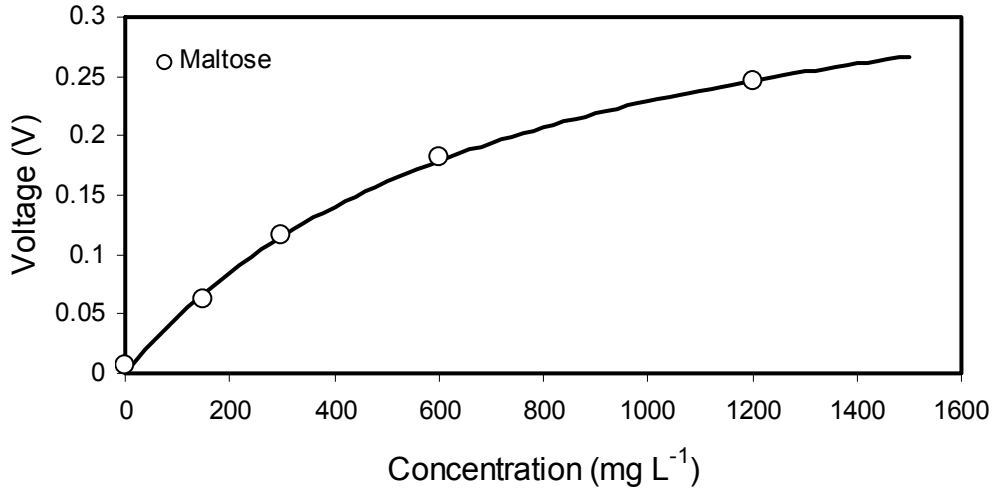

Figure 3.19: Effects of maltose concentrations on voltage output.

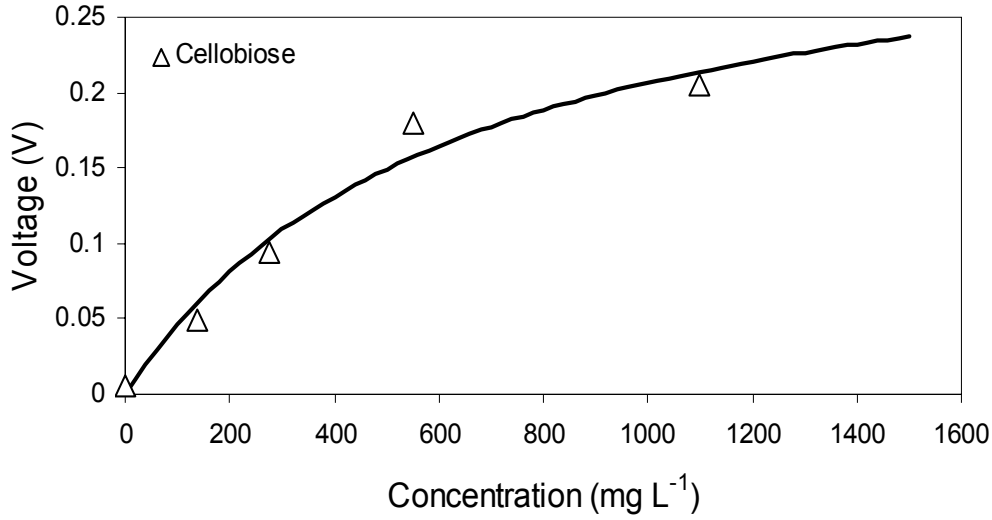

Figure 3.20: Effects of maltose concentrations on voltage output.

Table 3.2. Comparison of MFC performance by the monosaccharides and disaccharides.

	Substrate	Power density (mW m^{-2})	Ec (%)	COD Removal (%)	OD_{600nm} [b]	V_{max} (V)	Ks (mg L^{-1})	R^2 (%)
Monosaccharide	Glucose	2164±2	28	93±2	0.245	0.39	637	0.993
	Xylose	2331±62	31	95±2.1	0.131	0.38	352	0.960
	Glucuronic acid	2767±27	24	89±0.8	0.207	0.44	725	0.987
Disaccharide	Maltose	1893±67	30	82±12	0.201	0.40	733	0.998
	Cellobiose	1262±5	18	81±1	0.379	0.34	626	0.971

3.3 Electricity Generation from Polyalcohols

3.3.1 Voltage output and adaptation time with polyalcohols

When a stable power output was generated using sodium acetate as carbon source, the culture medium was replaced with a medium containing one of the polyalcohols.

While all of the polyalcohols tested produced electricity, the adaptation times varied with each polyalcohol (Fig. 3.21-3.26). The bacteria on the anode most easily adapted to mannitol and started to produce stable voltage around 7 hours after the addition of the mannitol medium solution (Fig. 3.25). While the adaption times were similar for ribitol (~16 h) (Fig. 3.23), sorbitol (~17 h) (Fig. 3.26) and galactitol (~22 h) (Fig. 3.24), it took longer time for the bacteria to produce stable voltage from arabitol (~45 h) (Fig. 3.22) and xylitol (~70 h) (Fig. 3.21). After achieving a stable voltage output with the polyalcohols, the same pattern continued in the consecutive batches using the same substrates. Current at 1k Ω varied depending on the polyalcohols chosen. The maximum current was produced by galactitol (0.56 mA), while mannitol resulted in the lowest current (0.24 mA). Ribitol, xylitol, sorbitol, and arabitol produced 0.32 mA, 0.29 mA, 0.26 mA and 0.26mA, respectively.

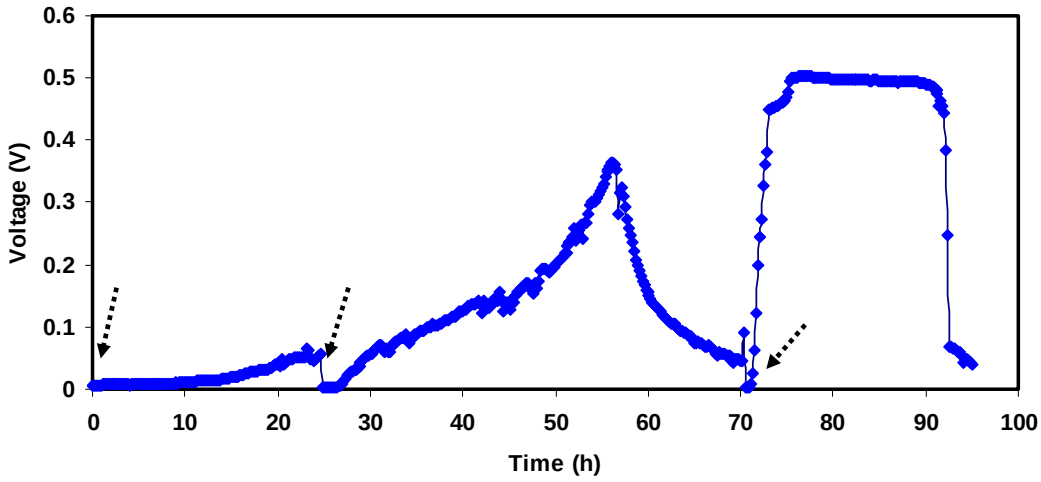

Figure 3.21: Electricity generation from xylitol, using a mixed bacterial culture at 1k Ω resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

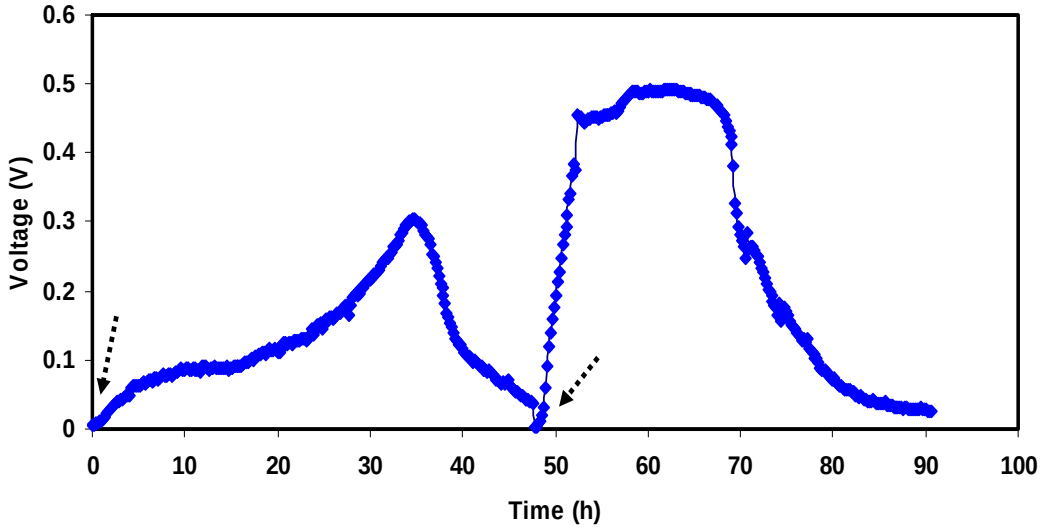

Figure 3.22: Electricity generation from arabitol, using a mixed bacterial culture at 1k Ω resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

Ribitol produced electricity in air-cathode single chamber MFC (Figure 3.23).

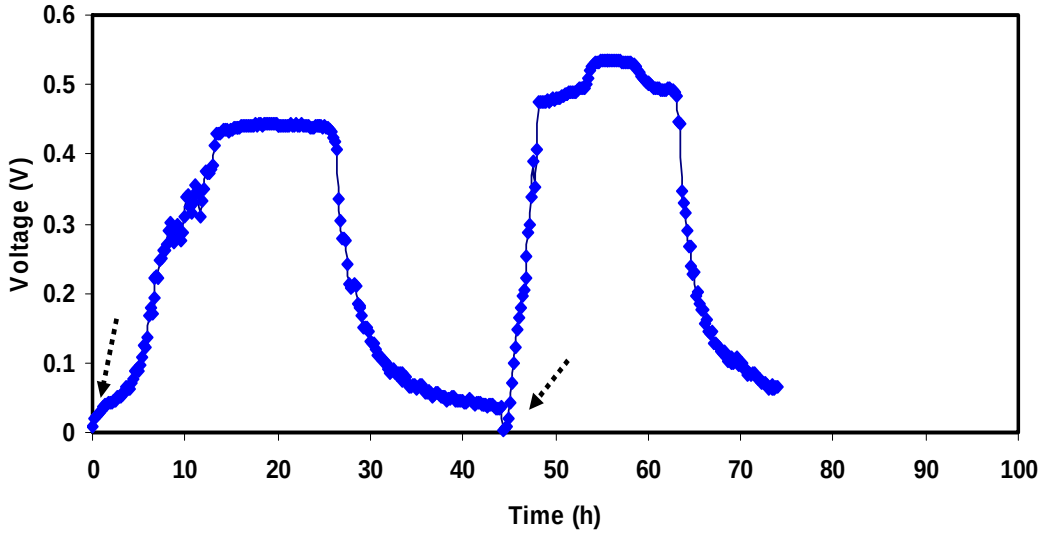

Figure 3.23: Electricity generation from ribitol, using a mixed bacterial culture at 1k Ω resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

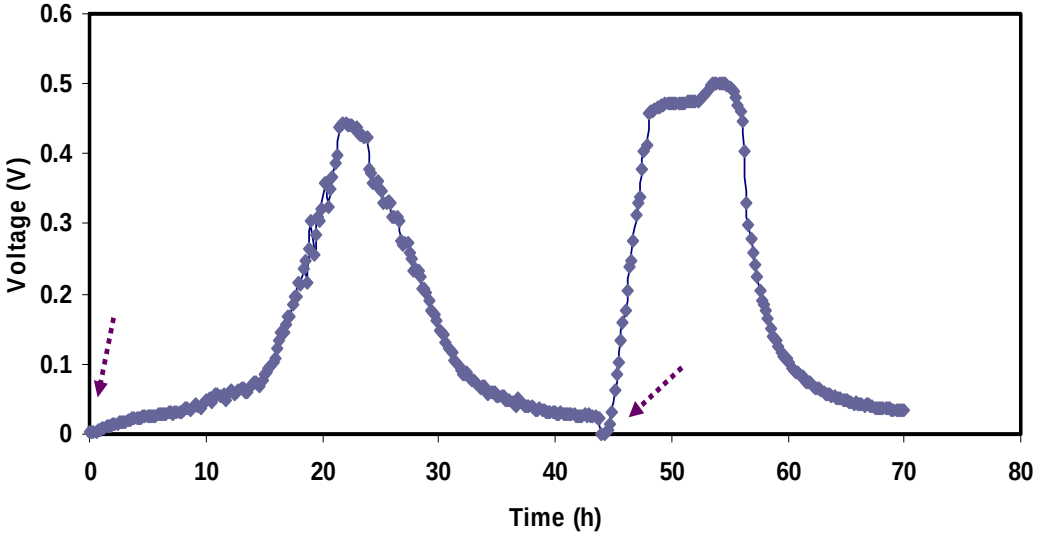

Figure 3.24: Electricity generation from galactitol, using a mixed bacterial culture at 1k Ω resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

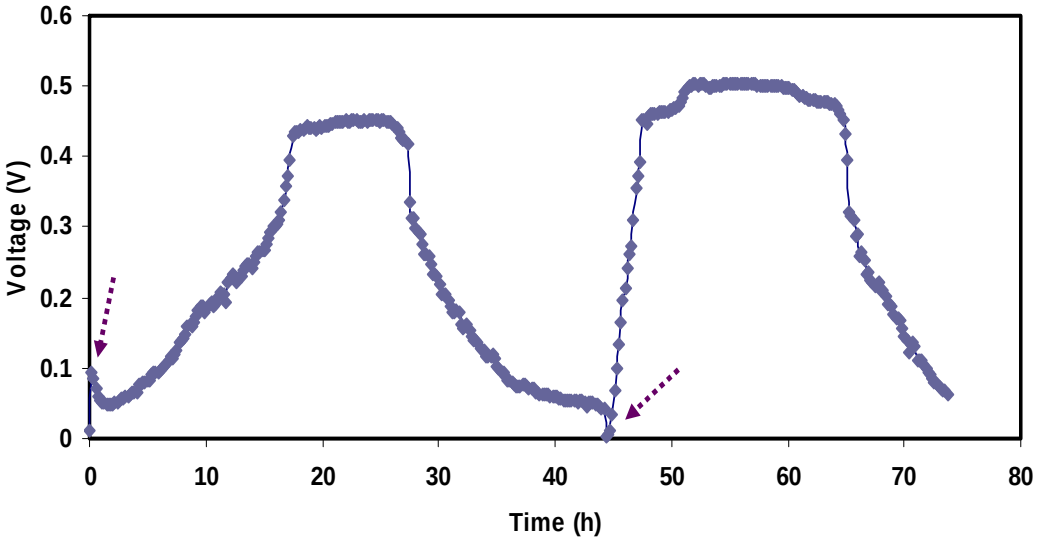

Figure 3.25: Electricity generation from mannitol, using a mixed bacterial culture at 1k Ω resistance.

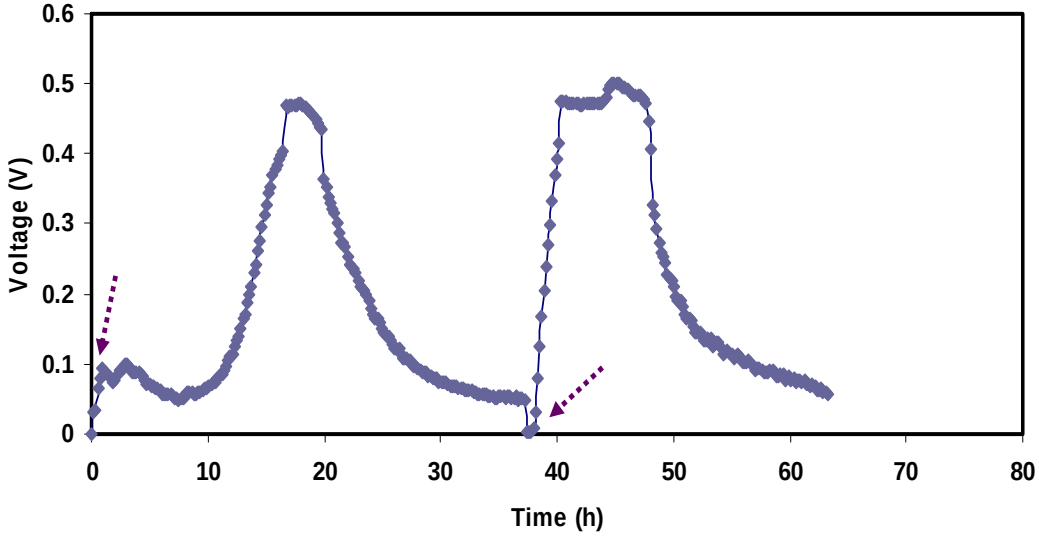

Figure 3.26: Electricity generation from sorbitol, using a mixed bacterial culture at 1k Ω resistance. Arrows indicate the replacement of the culture media with a fresh medium solution.

3.3.2 Power generation in the presence of polyalcohols

The maximum power density generated in MFCs with each polyalcohols was determined by varying the circuit resistance from 50 to 1k Ω (Fig. 3.27). Among the three pentitols tested, ribitol generated a maximum power density of 2347±141 mW/m^2 and xylitol and arabitol generated 2107±84 mW/m^2 and 2027±117 mW/m^2, respectively. Among the three hexitols, sorbitol generated the highest power density of 2653±12 mW/m^2 at a current density of 0.78 mA/cm^2 followed by galactitol (1691±84 mW/m^2). Mannitol generated the lowest power density of 1488±160 mW/m^2 at a current density of 0.55 mA/cm^2 (Fig. 3.27, Table 3.2). The power densities generated in this study are comparable to the power densities generated using various monosaccharides (1407-2763 mW/m^2 at 0.76-1.18 mA/cm^2) using the same MFC configuration (Catal *et al.*, 2008a).

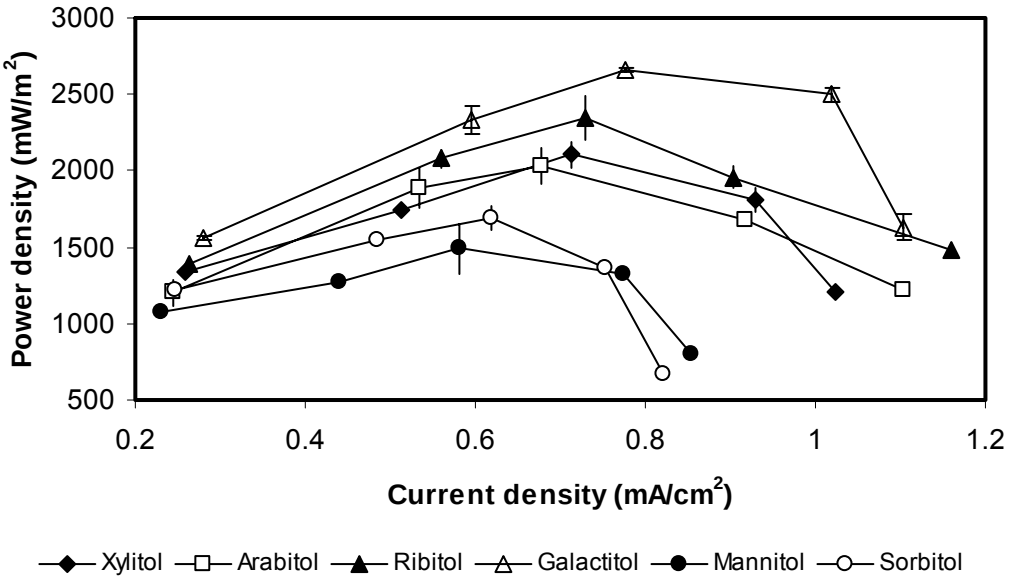

Figure 3.27: Power density as a function of current density obtained from pentitols.

3.3.3 Effect of polyalcohol concentration

The effect of polyalcohol concentrations on electricity generation at 120 Ω external resistances was determined (Table 3.3, Fig. 3.28). Maximum voltage ranged from 0.24 V to 0.34 V and half saturation constant (Ks) ranged from 298 mg/L to 753 mg/L. Galactitol produced the highest maximum voltage (0.34 V) with Ks=437 mg/L and mannitol produced the lowest voltage (0.24 V) with Ks=572 mg/L among the polyalcohols examined. Our results showed that half saturation constant was strongly dependent on substrate type, which was also found in our previous study using various monosaccharides as substrates (Catal *et al.*, 2008a). The half saturation constant was also dependent on the external resistance used. For example, a much lower Ks (43 mg/L) was obtained at 1k Ω compared to the Ks (141 mg/L) at 218 Ω (Liu *et al.*, 2005). Experiments conducted around an external resistance corresponding to the maximum power output will allow us to have a better evaluation of the effect of substrate concentration on MFC operations since MFCs are preferred to be operated at an external load with a maximum power output.

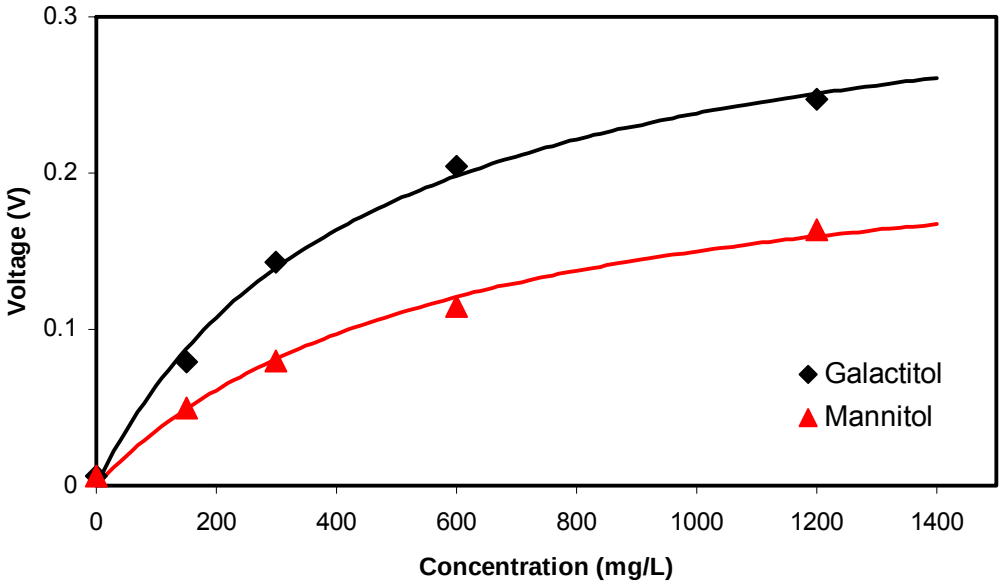

Figure 3.28: Effects of galactitol and mannitol concentrations on voltage output.

3.3.4 Chemical oxygen demand removal and coulombic efficiency results with polyalcohols as substrates

The COD removal for polyalcohols was in the range of 71-91% (Table 3.3). The Coulombic efficiency, which was calculated based on the total substrate concentration, however, was in the range of 10-19% for hexitols and 21-28% for pentitols at 120 Ω (Table 3.3). The significant electron loss was possibly due to the electron transfer from polyalcohols to other electron acceptors in solution, such as oxygen diffused through the air cathode, and the substrate utilization for bacterial growth (Liu *et al.,* 2005). The lower Coulombic efficiencies obtained with hexitols was possibly because hexitols could be utilized by larger variety of microorganisms than pentitols (London and Chace, 1977) and resulted in faster bacterial growth (substrate utilization) of non-exoelectrogens in solutions and on electrodes. This was evidenced by the fact that higher cell densities (OD_{600}) were observed in the solutions with hexitols (Table 3.3), and there were diverse bacterial species observed on the anodes of MFCs fed with hexitols.

Medium solution pH did not change significantly during the batch operation and ranged from 6.8 to 6.9 from an initial pH of 7.0.

Table 3.3. MFC performance using different polyalcohols as carbon sources.

Polyalcohol	Power density[a] (mW/m^2)	$OD_{600\ nm}$	CE[b] (%)	COD (%)	V_{max} (V)	Ks (mg/l)	R^2 (%)
Galactitol	2650±12	0.289	13	90±5	0.34	437	0.983
Ribitol	2347±141	0.152	28	92±1	0.32	753	0.936
Xylitol	2107±84	0.070	21	91±2	0.29	705	0.956
Arabitol	2027±117	0.188	25	91±1	0.26	561	0.962
Sorbitol	1691±84	0.204	10	71±1	0.26	298	0.953
Mannitol	1488±160	0.258	19	91±1	0.24	572	0.997

[a] Mean value (n=2) ± S.E. [b] Coulombic efficiencies were calculated according to 120 Ω resistance using 7 cm^2 anode.

The results above demonstrate that all of the polyalcohols tested could produce electricity in air-cathode MFCs. Although only a limited number of bacteria and fungi are able to utilize pentitols as growth substrates (London and Chace, 1977), our mixed bacterial culture efficiently utilized all the tested polyalcohols. This mixed culture also demonstrated power generation from acetate (Fan *et al.,* 2007) and 12 monosacchrides in our previous study (Catal *et al.,* 2008a). For many electricity generating bacteria, only certain substrates can be used as carbon sources. For example, carbon sources for many *Geobacter* species are primarily limited to simple organic acids and aromatic compounds (Lovley, 2006b; Bond and Lovley, 2003). The *Pseudomonas* species isolated from an MFC with glucose as a carbon source could not further utilize the fermentative products, such as acetate, for electricity production (Rabaey *et al.,* 2004). Most wide type *Shewanella* species could only incompletely oxidize a limited number of organic acids such as lactate and pyruvate to acetate under anaerobic conditions (Lovley, 2006). Depending on the species, electron transfer mechanisms vary, and also determine the type of MFCs if they are mediator-utilizing or mediator-less. Up to now, three different electron transfer mechanisms from microorganisms (i. bacterial nanowires; ii. electron transfer by cell-surface proteins; ii. chemical mediators) have been reported. The role of bacterial nanowires in electro transfer has been demonstrated in *Shewanella* species (Gorby *et al.,* 2006). *Geobacter sulfurreducens* has been shown that outer-membrane

cytochromes, might play a role in electron transfer from microorganisms to electrodes (Magnuson, 2001). Direct electron transfer from yeast cells was demonstrated in mediator-less MFCs (Prasad *et al.,* 2007). Zhang *et al.,* showed that electron transfer between electrode and *E. coli* cells is carried out by soluable compounds in the culture (Zhang *et al.,* 2008). Yet, *Pseudomonas aeruginosa* has been reported as a mediator producer (phenazine) to stimulate electron transfer for several bacterial strains (Rabaey, 2004). Another, exoelectrogenic bacterium *Ochrobactrum anthropi* has been recently reported (Zuo *et al.,* 2008). In the current study, all of the substrates could be utilized by bacteria and the broad substrate utilization range could be explained by the use of mixed bacterial culture in MFCs. Although our community analysis using DGGE indicates the presence of over 9-12 bacterial species in the anode biofilms, depending on the polyalcohol species, it's still not clear about how many of them are exoelectrogens, and how they involved in the utilization of substrates and electron transfer process. Identifying the microbial community and culturing pure bacterial species are very important point to achieve higher MFC efficiency.

Although all the sugar alcohols tested are simple carbohydrates with molecules containing 5 or 6 carbons, their molecular structures are different. Previously, London and Chace (1977) demonstrated that pentitol phosphate pathway is analogous to the hexitol phosphate pathway found in strict or facultatively anaerobic bacteria. Its description completes the symmetry in the evolution of polyol disimilating pathways in that the existence of both phosphorylated and nonphosphorylated pathways for hexitol and pentitol utilization (London and Chace, 1977). For example, D-mannitol, D-sorbitol, and galactitol occur naturally and each of these can be utilized by *E. coli* K-12 as a total source of carbon and energy. Each hexitol enters the cell through a specific phosphotransferase system so the first intracellular species is the 6-phospho derivative. D-mannitol-1-phosphate is converted by a single dehydrogenase reaction to the glycolytic intermediate, D-fructose-6-phosphate, and thus, flows through the pathways of central metabolism to satisfy the cell's need for precursor metabolites, reducing metabolic energy, and power (Mayer and Boos, 2005). Pentitols are transported into the cell by a phosphoenolpyruvate phosphotransferase system that converts them to pentitol phosphate, whereupon a specific dehydrogenase oxidizes the intermediate product

ketopentose phosphate which is finally converted to xylulose-5-P, and enters into central metabolism (London and Chace, 1977). These differences in structure may be attributed to the selection of bacterial species on anode (or cathode) and/or to different metabolic pathways even for the same bacterial species, resulting in the difference in power density, adaption time and Coulombic efficiency in MFCs. Higher Coulombic efficiencies (40-55%) were previously reported using the same air- cathode MFC system fed with glucose in the presence of proton exchange membrane, (Liu and Logan, 2004). On the other hand, coulombic efficiency of 83% was previously reported using glucose-fed mediator-less two chamber system (Chaudhuri and Lovley, 2003). Dissolved oxygen in the system might significantly limit the cathode reaction (Gil *et al.,* 2003). If diffused/dissolved oxygen is limited in the system, perhaps higher Coulombic efficiency could be achieved. Although all the sugar alcohols tested are simple carbohydrates with molecules containing 5 or 6 carbons, their molecular structures are different. These differences in structure may conttribute to selection of bacterial species on anode (or cathode) and/or to different metabolic pathways even for the same bacterial species, resulting in the difference in power density, adaption time and Coulombic efficiency in MFCs.

Polyalcohols are by-products in ethanol fermentation process. Certain amount of polyalcohols were also found in the water soluble materials of corn stover (3% to 7%), which accounted for about 14-27% of the total biomass on a dry weight basis (Chen *et al.,* 2007). An effective use of polyalcohols could also constitute an important step of utilizing biomass for economical production of renewable energy. In combination with our previous findings that monosacharides from lignocellulosic biomass were good carbon sources electricity-generating bacteria, we have demonstrated that efficient utilization of lignocellulosic carbohydrates in the forms of monosacharides and polyalcohols in MFCs for electricity generation was indeed possible, which has great implications on the economical production of renewable energy from lignocellulosic materials with the MFC technology (Catal *et al.,* 2008c).

3.4 Effects of Furan Derivatives and Phenolic Compounds on Voltage Generation

3.4.1 Voltage generation using selected compounds as the sole carbon sources

All the test compounds were selected based on their potential presence in an acid-pretreatment or acid-hydrolysis of lignocellulosic biomass. These compounds were individually tested as the sole carbon sources for electricity production in MFCs. A voltage of 0.46 V was produced after the solution was replaced with the 10 mM 5-HMF solution but quickly decreased to below 0.4 V. In the subsequent batches, i.e, when the MFC solution was replaced with a fresh glucose-free medium containing the same amount of 5-HMF, the voltage decreased to 0.12 V (Fig. 3.29). None of the other compounds tested were able to produce electricity in the absence of the additional carbon sources (data not shown).

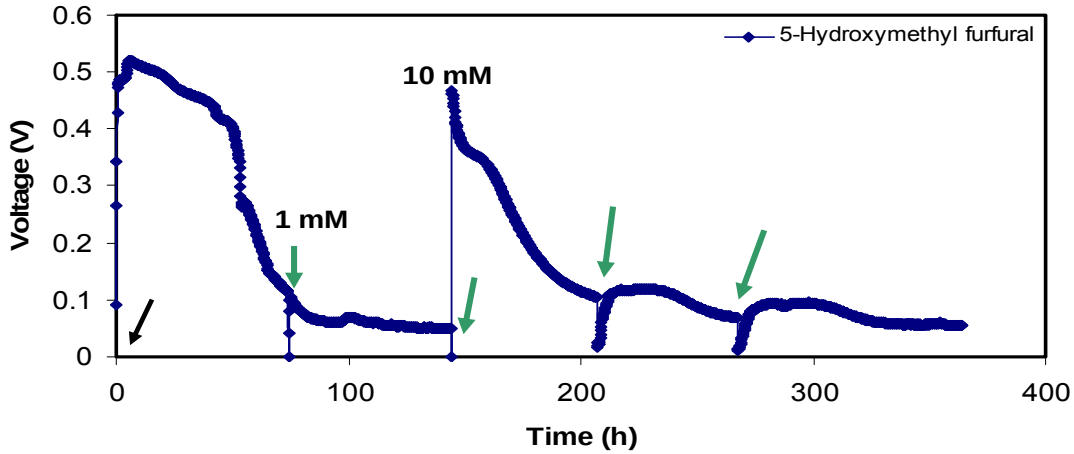

Figure 3.29: Electricity generation from 5-HMF in the absence of carbon sources. Black arrow indicates the addition of glucose-containing medium and green arrows indicate the addition of 5-HMF containing medium solution.

3.4.2 Influence of 5-HMF, vanillin, trans cinnamic acids and 3,5-dimethoxy-4-hydroxy- cinnamic acid on voltage generation using glucose

The maximum voltage output was not affected by 5-HMF at a concentration of up to 10 mM and decreased only about 12% when the HMF concentration was increased from 10 mM to 40 mM (Fig. 3.30A). Voltage was quickly recovered to the original level after replacing the solution with a glucose-containing medium without 5-HMF

(Fig. 3.30A). Similar effects on voltage generation were observed for vanillin, *trans*-, and 3,5-dimethoxy-4-hydroxy-cinnamic acids except that the maximum voltage decreased about 25% for *trans*- cinnamic acid when its concentration was increased from 20 to 40 mM (Fig. 3.30B, C, D).

5-HMF is formed by dehydration of hexoses such as glucose, during the pretreatment of biomass (Palmqvist and Hagerdal, 2000) and can inhibit ethanologenic microorganisms such as yeasts, at a concentration of ~ 8-120 mM (1-15 g L^{-1}) depending on the strain used (Almeida *et al.,* 2007). The concentration of 5-HMF was generally low when steam explosion pretreatment was applied and it ranged from 0.47 mM (0.06 g L^{-1}) to 4.7mM (0.6 g L^{-1}) in the hydrolysates of sugar cane, corn stover and poplar (Martin and Jonsson, 2003; Ohgren *et al.,* 2006). These concentrations were much lower than 40 mM, at which the electricity generation was affected in MFCs. However, higher concentrations of 5-HMF 16 - 46 mM (2-5.9 g L^{-1}) were detected in the hydrolysate of spruce when one- or two-step dilute acid hydrolysis was performed (Almeida *et al.,* 2007; Oliva *et al.,* 2003). Cinnamic acids can also be found in the hydrolysate of lignocellulosic biomass (Klinke *et al.,* 2002). It was reported that the cinnamic acid concentration was about 0.007 mM (0.001 g L^{-1}) after dilute acid hydrolysis of spruce (Klinke *et al.,* 2002). However, these concentrations were significantly lower than the 20 mM, at which could inhibit voltage generation in MFCs (Table 3.4).

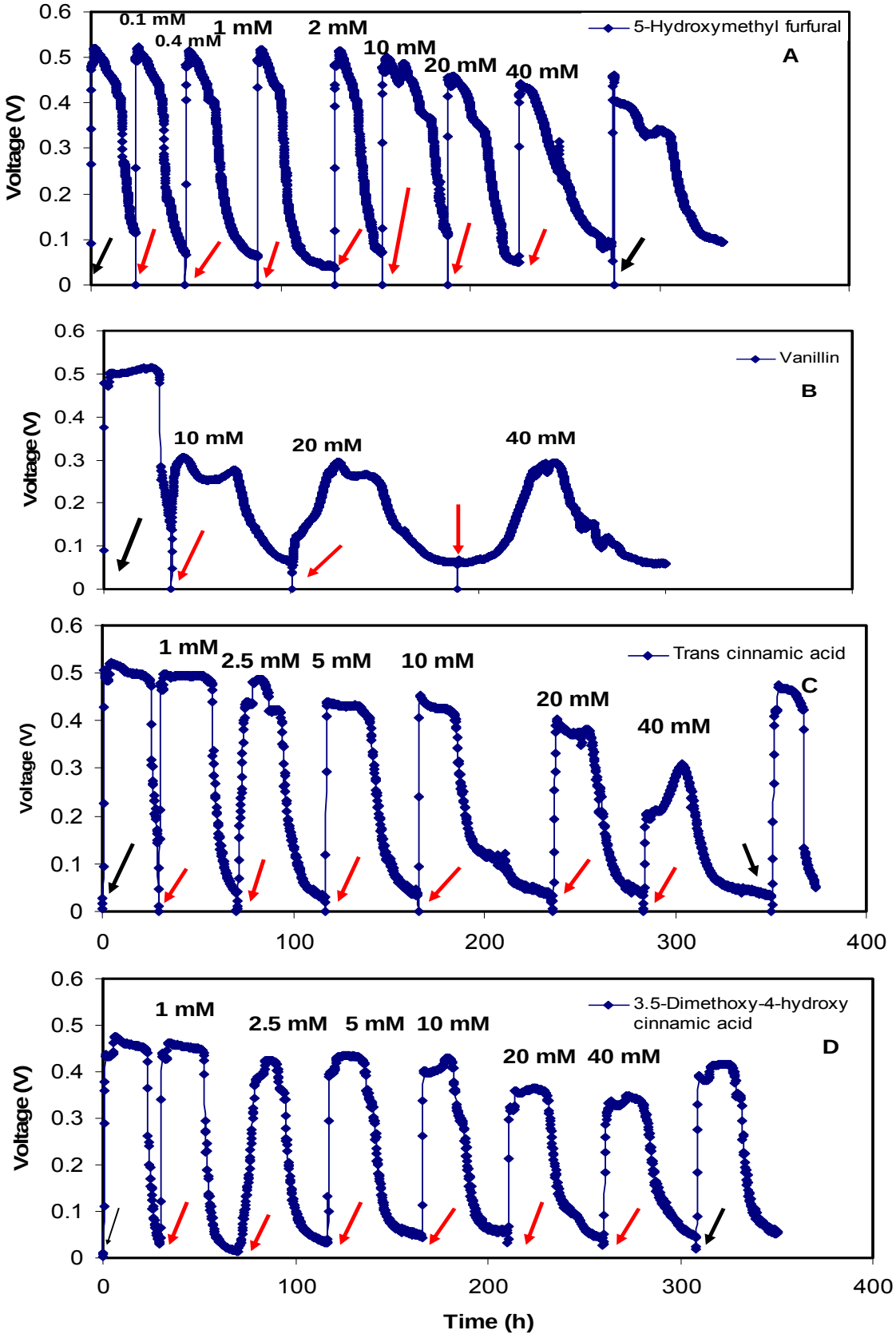

Figure 3.30: Influence of 5-HMF (A), vanillin (B), *trans*-cinnamic acid (C), 3,5-dimethoxy-4-hydroxy cinnamic acid (D) on voltage generation. Black arrows indicate the addition of glucose-containing medium and red arrows indicate the addition of medium solution containing both glucose and the selected compounds.

3.4.3 Influence of syringaldeyhde, trans-4-hydroxy-3-methoxy cinnamic acid, and 4-hydroxy cinnamic acid on voltage generation using glucose

These compounds did not affect electricity generation at lower concentrations, i.e., up to 2.5-5 mM, but severely inhibited the voltage output at higher concentrations (20 mM). At a concentration of 5 mM, syringaldehyde did not significantly affect the maximum voltage detected in comparison to that of the control. Voltage output was strongly inhibited by the addition of 20 mM syringaldeyde and reduced to 0.09 V (Fig. 3.31A). Similar results were observed for *trans*-4-hydroxy-3-methoxy acid (Fig. 3.31B, C). While a voltage of 0.44 V with the addition of 10 mM 4-hydroxy cinnamic acid, nearly no voltage was produced when its concentration increased to 20 mM. Voltage production was not recovered by subsequent replacement of medium solutions with fresh ones lacking these phenolic compounds (Fig. 3.31).

Syringaldeyde and vanillin can be formed upon degradation of syringyl propane units and guaiacylpropane units of lignin (Jonsson *et al.,* 1998). It was reported that vanillin and syringaldehyde concentrations were around 0.8 mM (0.12 g L^{-1}) and 0.6 mM (0.107 g L^{-1}), respectively in the solution after dilute acid hydrolysis of spruce (Larsson *et al.,* 2001), 0.18 mM (0.032 g L^{-1}) and 0.16 mM (0.024 g L^{-1}), respectively after wet oxidation hydrolysis of wheat straw (Klinke *et al.,* 2002), and 0.27 mM (50 mg L^{-1}) and 0.09 mM (14 mg L^{-1}), respectively in the steam exploded poplar hydrolysates (Jonsson *et al.,* 1998). Their concentrations in poplar wood hydrolysate could be further decreased to 0.08-10 μM (0.014-1.82 mg L^{-1}) and 0.13-17 μM (0.02-2.65 mg L^{-1}), respectively using enzymatic treatment (Cantarella *et al.,* 2004). These concentrations obtained using various pretreatment and hydrolysis methods were all much lower than 10 mM, at which the voltage generation was inhibited, indicating that these compounds may not be a concern when the hydrolysates of lignocellulosic biomass were directly used in MFCs for voltage generation.

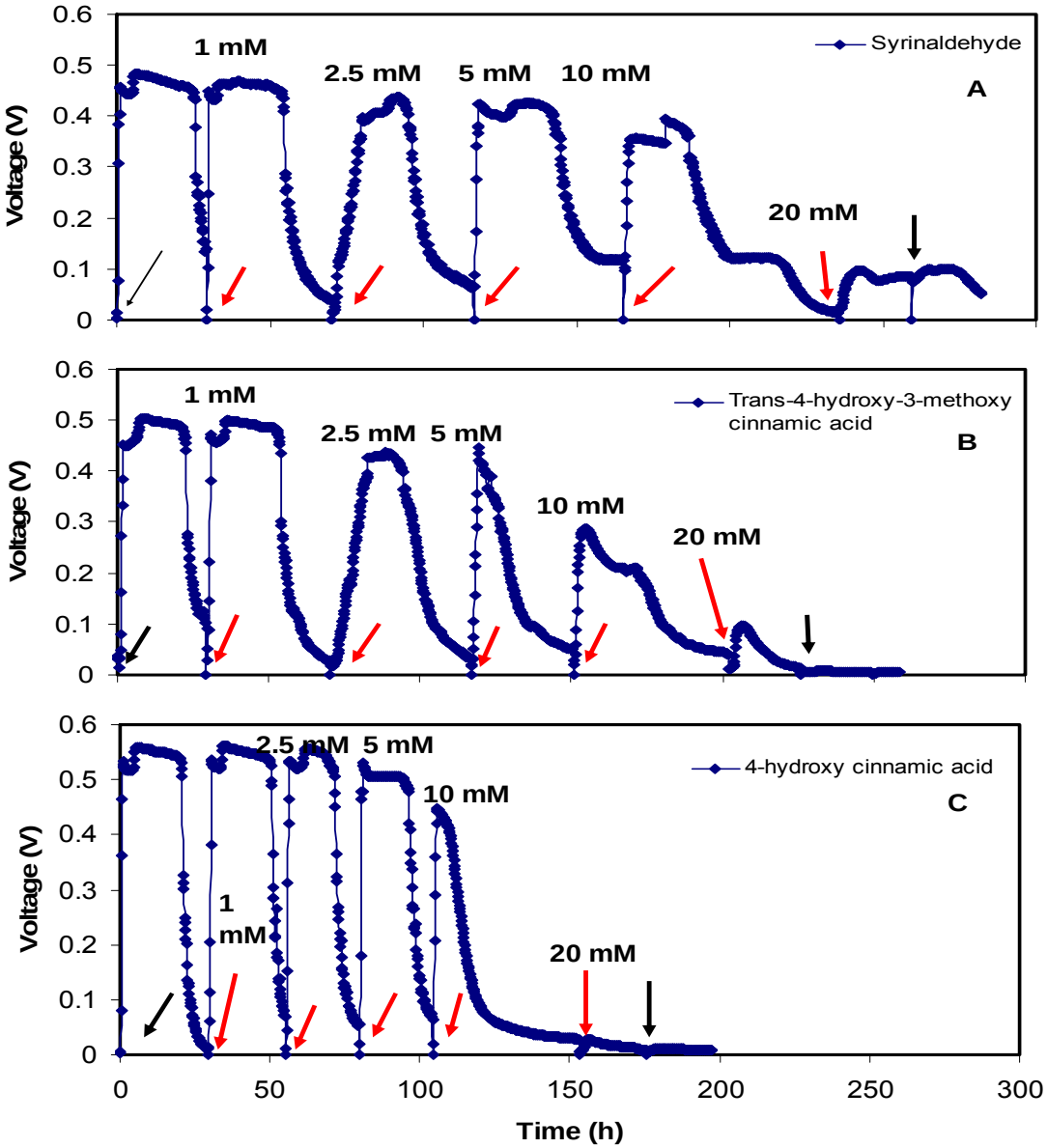

Figure 3.31: Influence of syringaldeyhde (A), *trans*-4-hydroxy-3-methoxy cinnamic acid (B), and 4-hydroxy cinnamic acid (C) on voltage generation. Black arrows indicate the addition of glucose-containing medium and red arrows indicate the addition of medium solution containing both glucose and the selected compounds.

3.4.4 Influence of furaldehyde, acetophenone, and 3,4-dimethoxybenzyl alcohol on voltage generation using glucose

MFC performance was affected significantly by the presence of 2-furaldehyde, acetophenone, and 3,4-dimethoxybenzyl alcohol even at a concentration less than 0.1 mM. While the voltage output was not affected by the addition of 0.01 mM 2-

furaldehyde, nearly 17% decrease in voltage production was observed when its concentration was increased to 0.05 mM. Following the MFC operation with 0.2 mM of 2-furaldehyde, voltage generation was not recovered upon the replacement of the medium with the glucose-containing furaldehyde-free medium solution (Fig. 3.32A). Acetophenone and 3,4-dimethoxybenzyl alcohol demonstrated inhibitory effects on, electricity generation at 0.05 mM and 0.1 mM, respectively (Fig. 3.32B, C). Voltage generation was also not obtained using glucose-containing medium when MFCs were operated in the presence of 0.2 mM acetophenone and dimethoxybenzyl alcohol solutions. The difference between figure 3.32 and other figures is because the replacement of the solution takes longer time.

Furaldehyde, a well known inhibitor for ethanologenic microorganisms, can be generated by acid hydrolysis of hemicellulose (Rao *et al.,* 2006). The concentration of 2-furaldehyde can be as high as 10 mM (1 g L^{-1}) in the hydrolysate of spruce when two stage dilute acid hydrolysis treatment was used (Larsson *et al.,* 1999). A lower concentration (~5.2 mM; 0.5 g L^{-1}) was reported when one step dilute acid treatment was used (Nilvebrant *et al.,* 2003). Benzyl alcohol was also found in the hydrolysate of steam explosion pretreated poplar with a concentration of ~6 mM (0.76 g L^{-1}) (Oliva *et al.,* 2003). The acetophenone concentration in the hydrolysate of wheat was ~0.03 mM (0.004 g L^{-1}) using alkaline wet oxidation method. The concentrations of these three compounds in the hydrolysates of biomass were either similar or higher than that at which inhibitory effects was observed in MFC, indicating that alternative or additional approaches should be employed to increase the efficiency of electricity generation from the hydrolysates of lignocellulosic biomass, such as: (1) use of appropriate pretreatment methods to yield low furan derivatives and phenolic compounds production, (2) removal of some strong inhibitors prior to the MFC process (Nilvebrant *et al.,* 2003), and (3) increasing the tolerance of bacteria towards these compounds through the enrichment of new bacterial cultures or genetic modification of the bacterial strains.

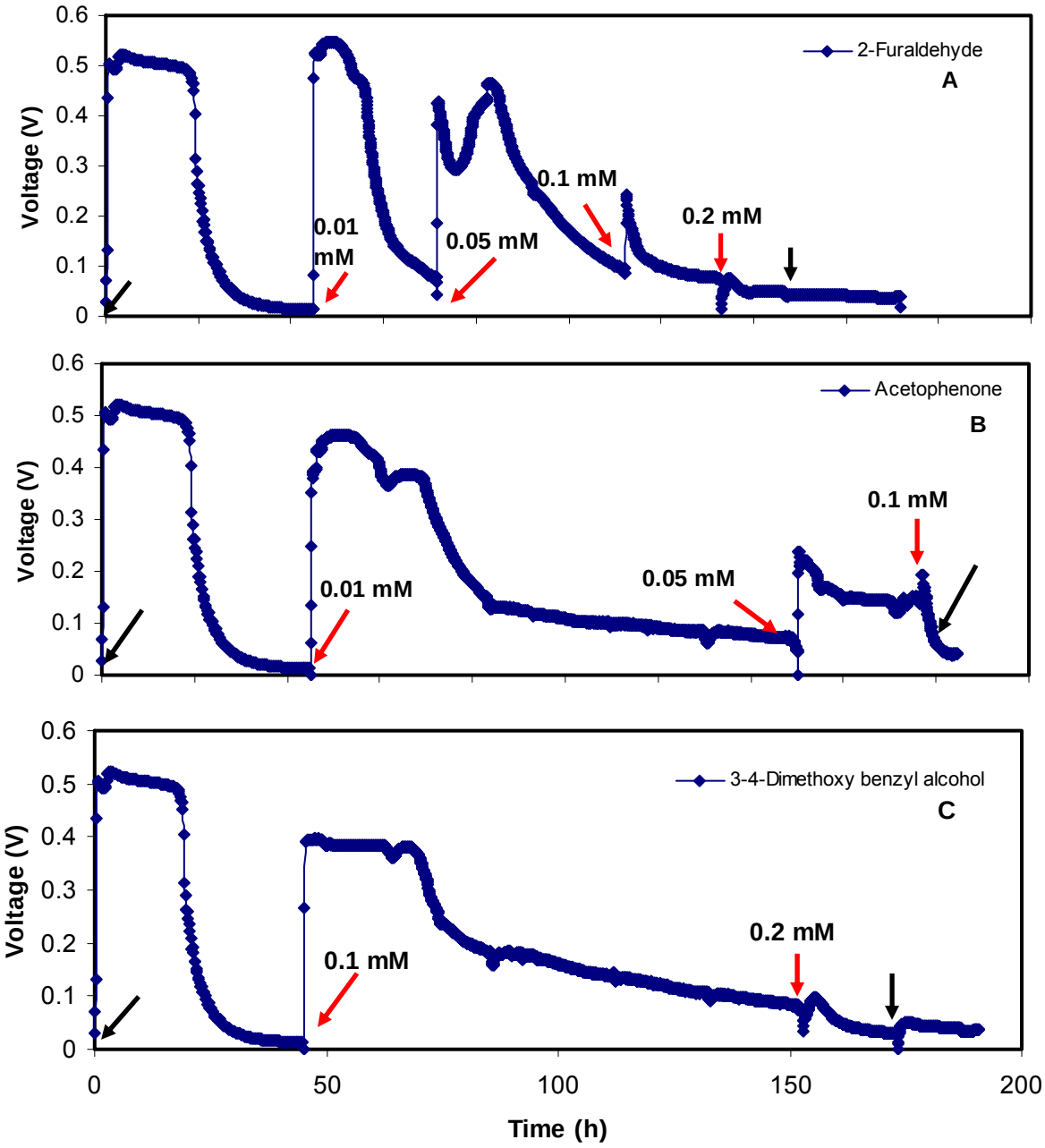

Figure 3.32: Influence of furaldehyde (A), acetophenone (B), and 3,4-dimethoxybenzyl alcohol (C) on voltage generation. Black arrows indicate the addition of glucose-containing medium and red arrows indicate the addition of medium solution containing both glucose and the selected compounds.

Table 3.4: Effect of examined compounds' concentrations on the voltage generated by bacterial culture in MFC, and comparision of the compounds found in wheat, spruce and poplar wood hydrolysates.

	Compounds	Voltage (V)			Concentrations (g/L)			
		0.1 mM	10 mM	20 mM	Wheat[1] (g/L)	Spruce[3] (g/L)	Poplar wood (mg/L)	
							Enz.[2]	No Enz.[3]
Group 1	Trans cinnamic acid	n.t.	0.43	0.38	n.i	0.01 (cinnamic acid or around 0.07 mM)	n.i	n.i.
Group 1	3-5-dimethoxy-4-hydroxycinnamic acid	n.t.	0.43	0.36	n.i	0.01 (cinnamic acid or around 0.07 mM)	n.i	n.i
Group 1	5-HMF	n.t.	0.50	0.45	n.i	5.9 (or ~0.46 mM); 3.2*	0.001-0.16	0.63
Group 2	Syringaldehyde	n.t.	0.39	0.10	0.024 (or ~0.01 mM)	0.107 (or ~0.6 mM)	0-0.45	0.27
Group 2	Vanillin	n.t.	0.35	0.27	0.032 (or ~0.2 mM)	0.12 (or ~0.8 mM)	0.0002-0.017	0.009
Group 2	Trans-4-Hydroxy-3-methoxy cinnamic acid	n.t.	0.29	0.01	n.i	0.01 (cinnamic acid or around 0.07 mM)	n.i	n.i
Group 2	4-Hydroxycinnamic acid	n.t.	0.45	0.02	n.i	0.01 (cinnamic acid or around 0.07 mM)	n.i	n.i
Group 3	2-Furaldehyde	0.12	n.t.	n.t.	n.i	1 (or ~10 mM), 1.5*	0.04-0.46	5.09
Group 3	Acetophenone	0.04	n.t.	n.t.	0.004 (or ~0.03 mM)	n.i	n.i	n.i
Group 3	3-4-dimethoxybenzyl alcohol	0.40*	n.t.	n.t.	n.i	n.i	n.i	4.5

[1]See ref. (Larsson *et al.,* 1999), See ref. (Klinke *et al.,* 2002) [2] See ref. (Cantarella *et al.,* 2004) [3] See ref. (Oliva *et al.,* 2003]; n.t. not tested.

* Inhibition was observed at 0.2 mM treatment

In conclusion, among the 2 furan derivatives and 8 phenolic compounds tested in this study, electricity was produced only from 5-HMF with a voltage output much lower than that using glucose. All the other compounds tested were unable to directly produce electricity in MFCs in the absence of other electron donors. When glucose was used as the carbon source, electricity generation was not significantly affected by the addition of 5-HMF, *trans*-, 3,5-dimethoxy-4-hydroxy- cinnamic acids at a concentration up to 10 mM while syringaldeyhde, vanillin, *trans*-4-hydroxy-3-methoxy, and 4-hydroxy cinnamic acids inhibited voltage generation at concentrations above 5 mM. Electricity generation was severely inhibited by 2-furaldehyde, acetophenone and 3-4-dimethoxybenzyl alcohol at concentrations smaller than 0.2 mM.

3.5 Consequences of Mixed-substrate Utilization in Microbial Fuel Cells

3.5.1 Voltage output obtained from sugar mixtures

Fig. 3.33-3.37 show the electricity generation from various sugar mixtures. The maximum voltage output was 0.56 V using glucose-galactose as substrates. Similar electricity generation pattern was observed in two-sugar combination (Fig. 3.33-37) while in all sugars combination experiment voltage could be produced for longer time due to the high sugar concentration (Fig. 3.37). This result indicates that sugar mixtures used in this study produce electricity in air cathode MFCs.

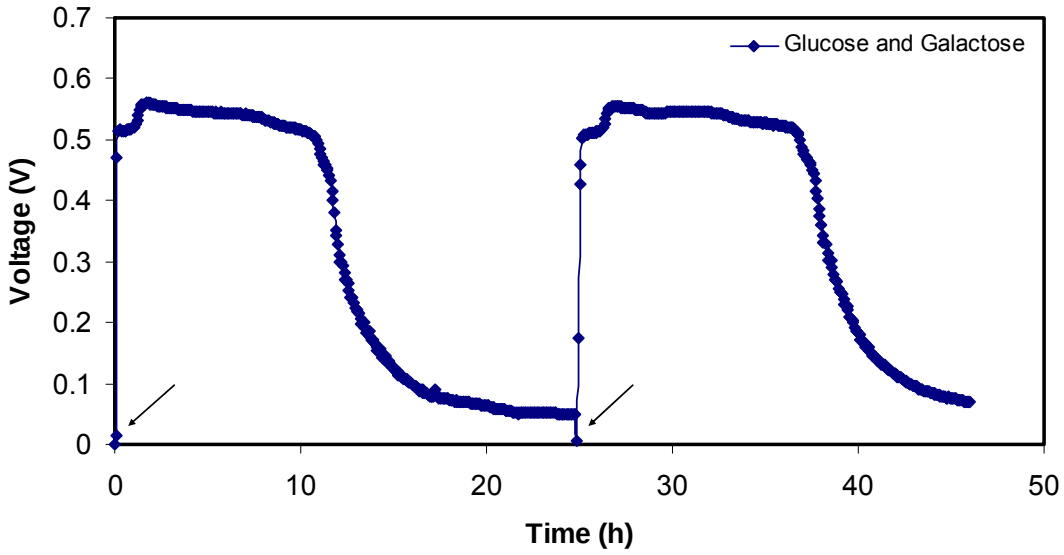

Figure 3.33: Voltage generation from glucose and galactose combination at 1k Ω external resistance.

The mixture of galactose and mannose produced electricity (Figure 3.34).

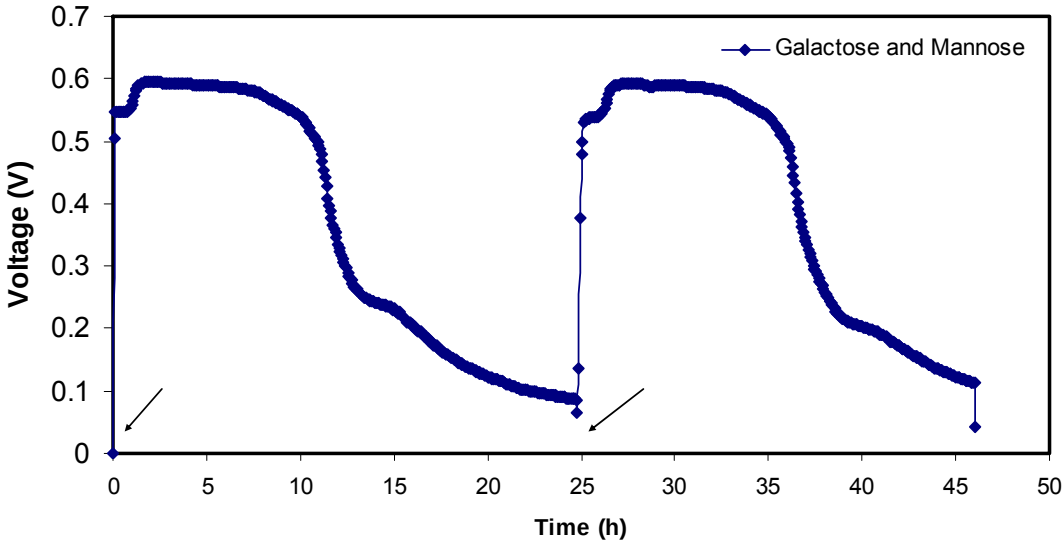

Figure 3.34: Voltage generation from galactose and mannose combination at 1k Ω external resistance.

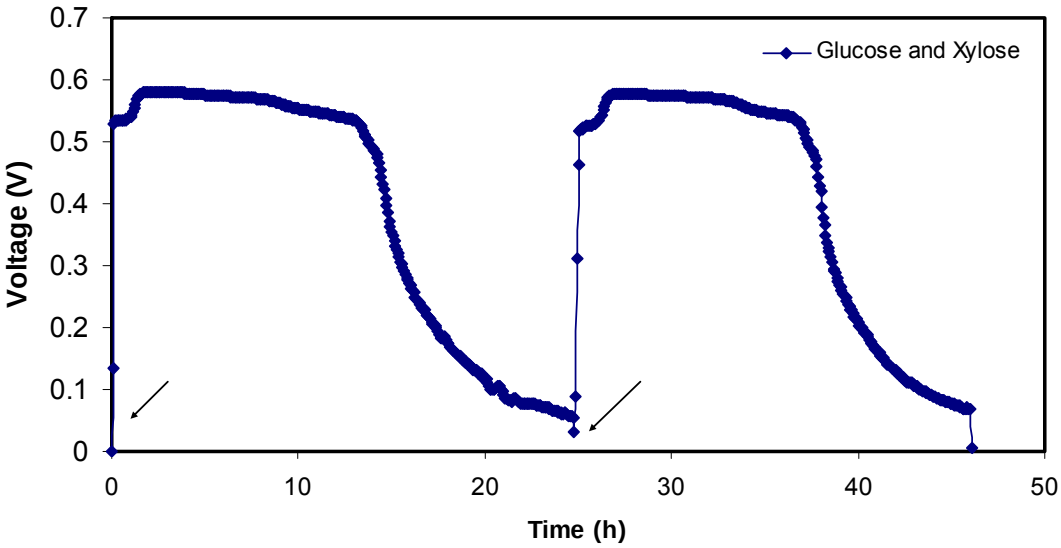

Figure 3.35: Voltage generation from glucose and xylose combination at 1k Ω external resistance.

The mixture of arabinose and xylose generated electricity in MFCs (Figure 3.36).

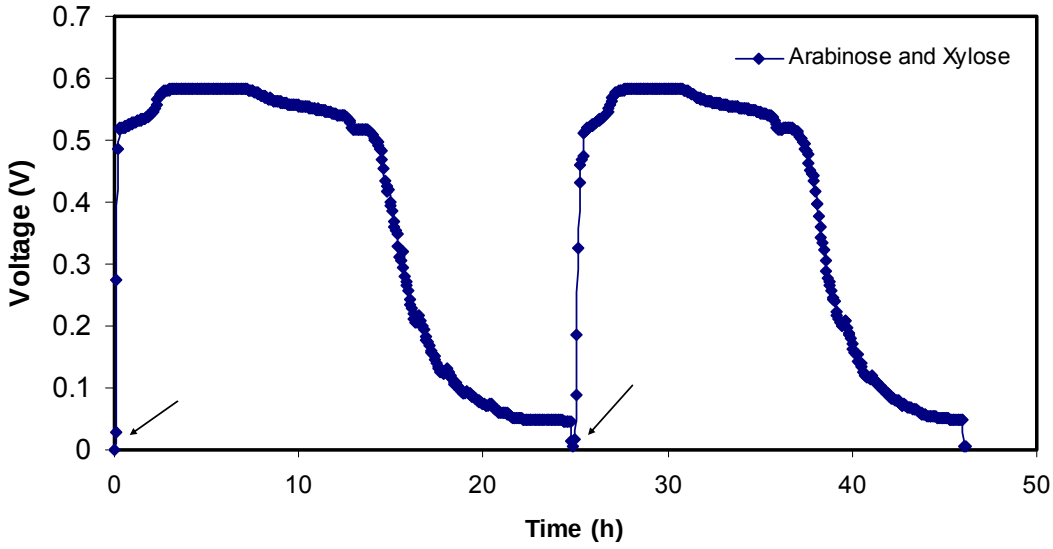

Figure 3.36: Voltage generation from arabinose and xylose combination at 1k Ω external resistance.

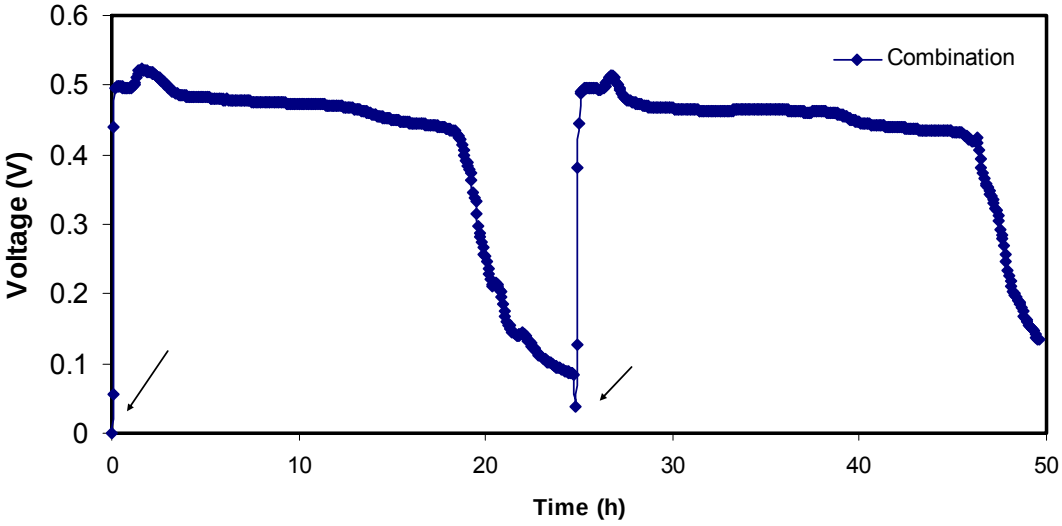

Figure 3.37: Voltage generation from glucose, galactose, mannose, arabinose and xylose combination at 1k Ω external resistance.

3.5.2 Substrate utilization patterns as a function of time

All sugars were utilized by the bacterial culture in MFC. To characterize sugar utilization patterns of the mixed bacterial culture, microorganisms were cultivated in medium with various sugar mixtures as described previously. The curves in figures 3.38-42 show the results obtained in sugar mixtures. In glucose-galactose medium, consumption of both sugars initially became detectable in 20 min, as observed in all other combinations as well. Glucose consumption was faster than galactose utilization (Fig. 3.38). In galactose-maltose medium, mannose was apparently preferred to galactose (Fig 3.39). In glucose-xylose medium, glucose was utilized faster than xylose (Fig. 3.40). In xylose-arabinose medium, the utilization of both sugars was simultaneous (Fig. 3.41). In the combination of all sugars, glucose and mannose utilization had similar pattern, while arabinose and galactose were consumed afterwards (Fig. 3.42).

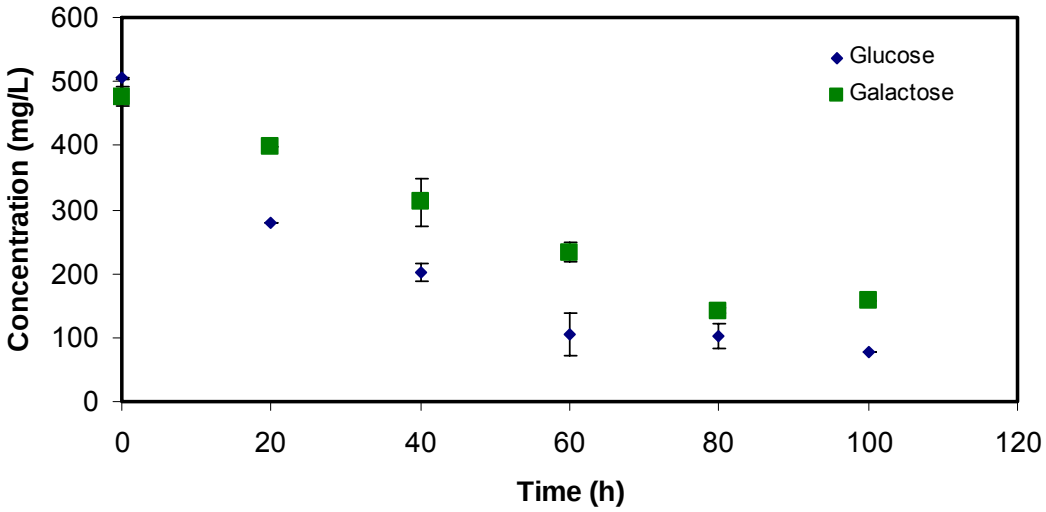

Figure 3.38: Utilization of the glucose-galactose mixture with time in the MFC.

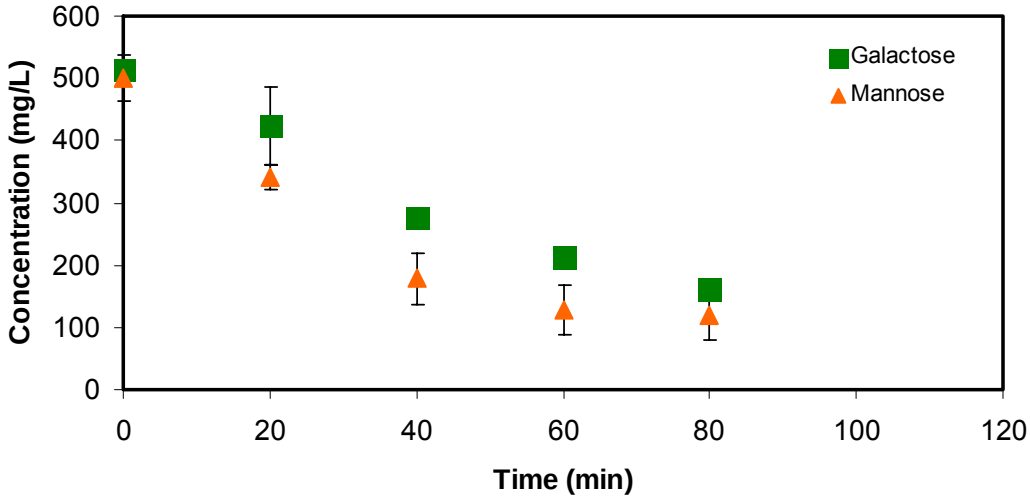

Figure 3.39: Utilization of the galactose-mannose mixture with time in the MFC.

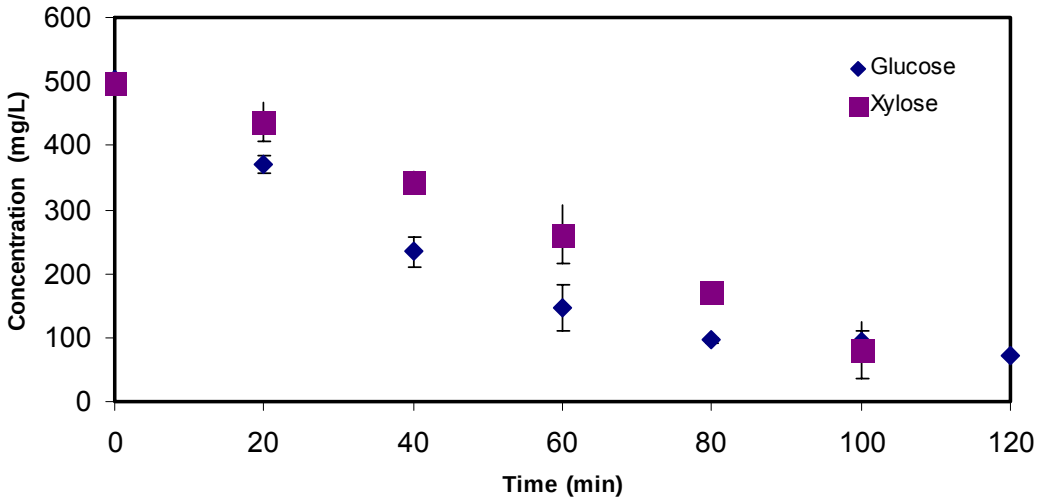

Figure 3.40: Utilization of the glucose-xylose mixture with time in the MFC.

The mixture of arabinose and xylose was utilized by microorganisms in MFCs (Figure 3.41).

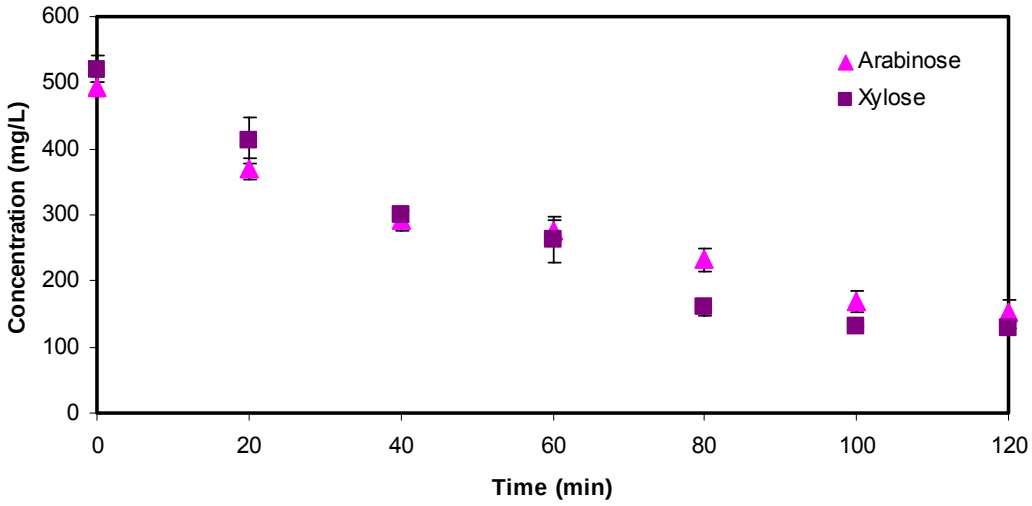

Figure 3.41: Utilization of the arabinose-xylose mixture over time in the MFC.

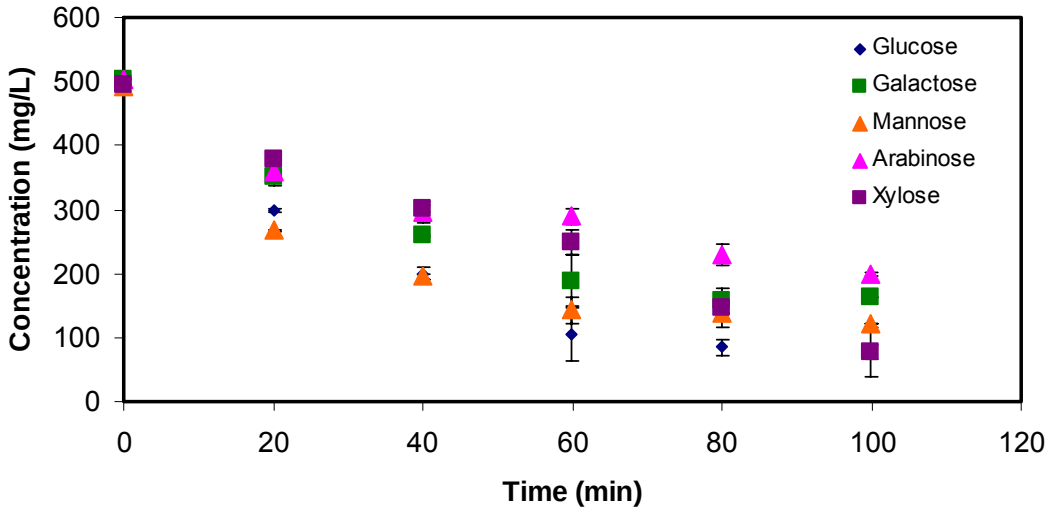

Figure 3.42: Utilization of the all sugar mixture with time in the MFC.

3.5.3 Carboxylic acid generation patterns in microbial fuel cells

We discovered that various carboxylic acids were produced when monosaccharides were used for electricity production (Fig 3.43-47). When a mixture of glucose and galactose was used as substrates, acetic acid, propionic acid, isobutyric acid, and isovaleric acid were produced. The concentration of propionic acid linearly increased from 0 to 100 min of operation, and then sharply decreased from then on (Fig 3.43). The concentration of acetic acid gradually increased from 0 to 120 min of the operation and then rapidly decreased from 120 min to 240 min. Propionic and acetic acids are two major carboxylic acids produced from a mixture of glucose and galactose. Isobutyric and isovaleric acids were detected but at very low concentrations. Propionic, acetic, and isobutyric acids were produced when a mixture of galactose and mannose was used as substrates. Propionic acid concentration linearly increased from 0 to 240 min of operation, and then decreased from 240 min to 480 min (Fig 3.44). The concentration of acetic acid gradually increased from 0 to 240 min of the operation and then rapidly decreased from 240 min on 480 min. Propionic acid and acetic acid are two major carboxylic acids produced from a mixture of galactose and mannose. Isobutyric acid was detected at very low concentrations. Propionic, acetic, and isobutyric acids were produced when a mixture of glucose and xylose was used as substrates. Similar concentration patterns were observed as seen in the mixture of glucose-galactose while isobutyric acid concentration was higher for the period of 0-100 min (Fig. 3.45). Only isobutyric and

propionic acids were detected in the operation of using the mixture of arabinose and xylose. The concentration of propionic acid gradually increased from 0 to 100 min of the operation and then rapidly decreased from 1200 min to 100 min while the concentration of isobutyric acid was stable for the period of 20-480 min (Fig 3.46). Propionic acid, acetic acid, and isobutyric acid were produced when a mixture of glucose-galactose-mannose-arabinose-xylose was used as substrates. Acetic acid concentration linearly increased from 0 to 120 min of operation, and then decreased from 120 min to 480 and 720 min (Fig 3.47). The concentration of propionic acid gradually increased from 0 to 240 min of the operation and then rapidly decreased from 240 min to 480 min (Fig 3.47).

In glucose-galactose medium, acetic acid and propionic acid concentrations increased up to 2.23, and 2.67 mM by 100 min, then decreased to 0.56 and 0 mM at 480 min, respectively. Isovaleric and isobutyric acids were also detected in glucose-galactose experiment (Fig 3.43). In galactose-mannose medium, propionic acid generation was lower when compared to glucose-galactose medium (Fig 3.44). The similar pattern of galactose-mannose medium was observed in glucose-xylose mixture (Fig 3.45). Propionic acid was increased through the initial 100 min of the run, then decreased, while isobutyric acid generation was almost the same throughout the 480 min in arabinose-xylose medium (Fig 3.46). In the combination of all sugars, acetic acid and propionic acids were observed for a longer period of time (Fig 3.47). However, the electricity generation started at around 25 h.

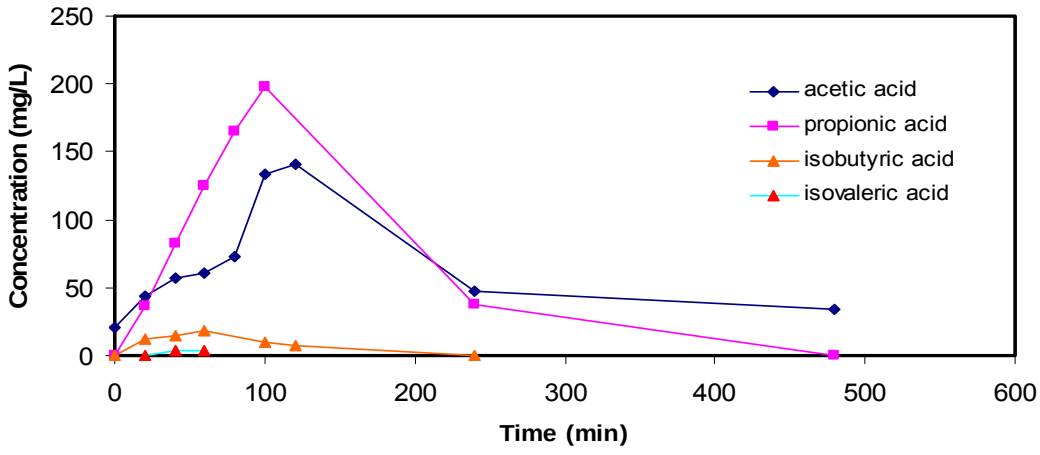

Figure 3.43: Carboxylic acids generated from the glucose-galactose mixture

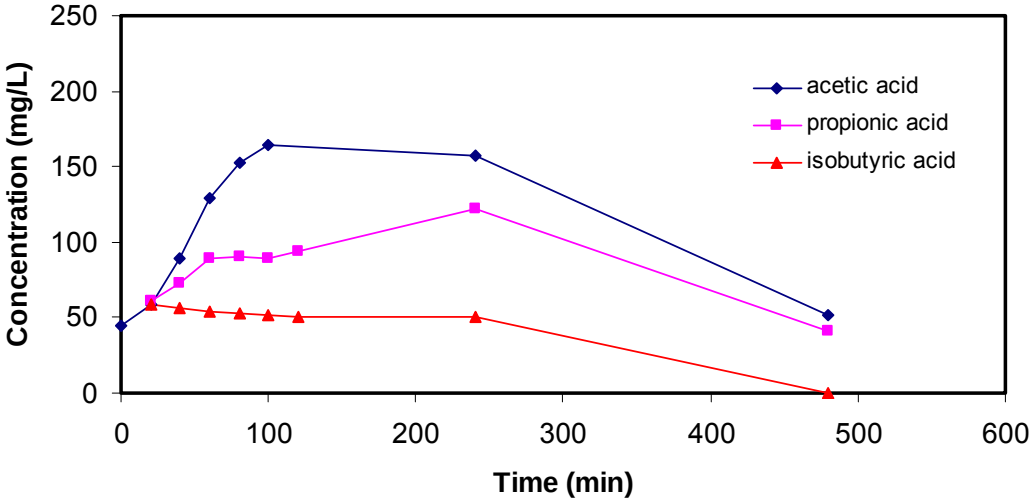

Figure 3.44: Carboxylic acids generated from the galactose-mannose mixture during MFC operation.

Acetic, propionic and isobutyric acids were produced during MFC operation (Figure 3.45).

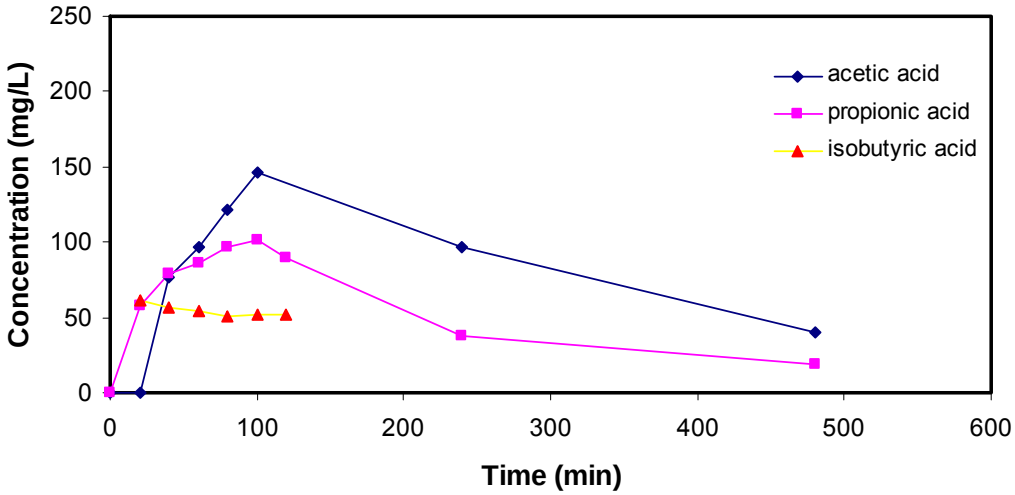

Figure 3.45: Carboxylic acids generated from the glucose-xylose mixture during MFC operation.

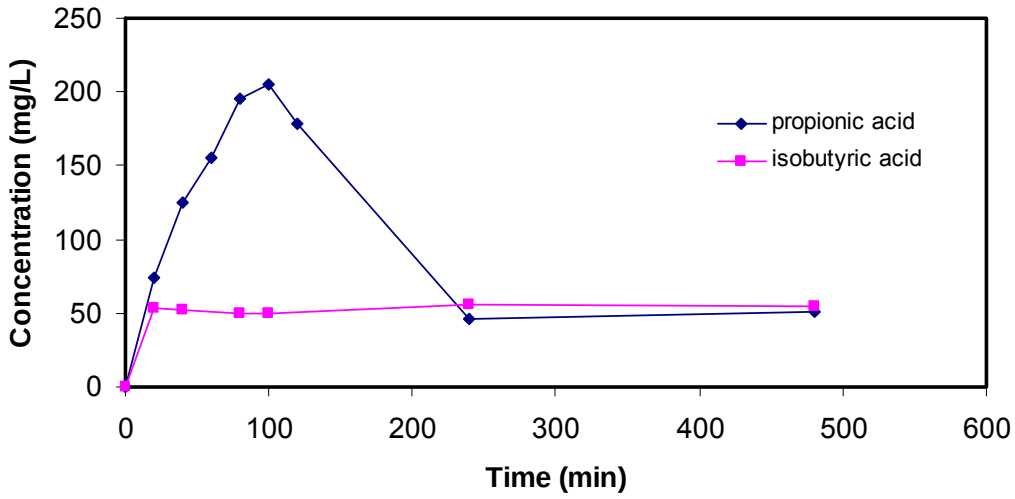

Figure 3.46: Carboxylic acids generated from the arabinose-xylose mixture during MFC operation.

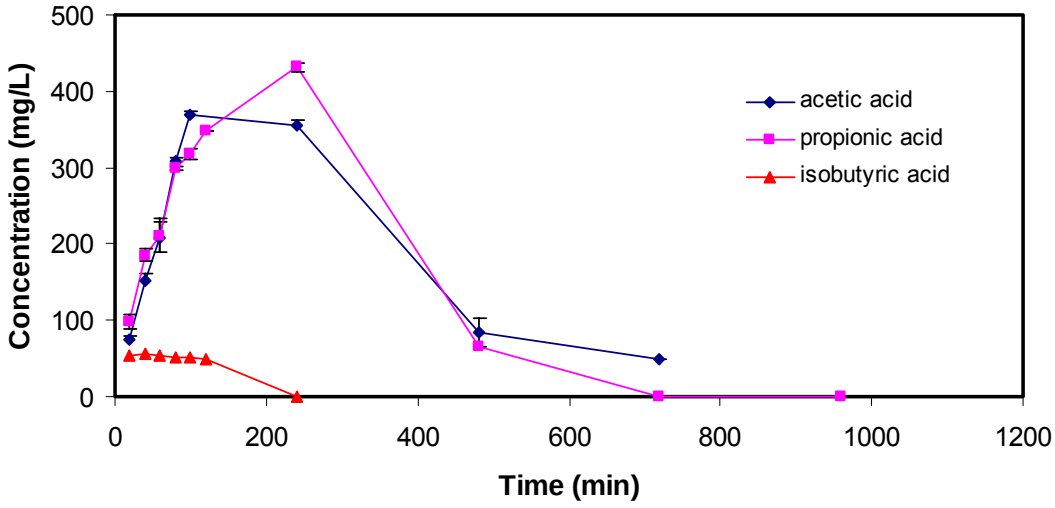

Figure 3.47: Carboxylic acids generated from the glucose-galactose-mannose-arabinose-xylose mixture during MFC operation.

The MFC technology needs to be improved for economically feasible applications and in this respect lignocellulosic biomass which contains high carbohydrate stock may help to decrease the process costs (Logan *et al.*, 2006; Catal *et al.*, 2008a). The scale-up of this technology needs to deal with some problems such as isolation of good-electricity producer microorganisms in the system (Liu *et al.*, 2008) and higher power output as well as better understanding of the substrate utilization patterns for process optimization. The operation for electricity production using lignocellulosic

biomass requires pretreatment methods and produces complex sugar mixtures of which the utilization patterns in MFCs are still unknown. Mixed-sugar utilization patterns were previously reported in many microorganisms, focusing particularly bioethanol production by yeasts. Our study showed that our bacterial cultures can simultaneously use all the sugars tested for electricity production, and it does not seem to have substrate affinity possibly because of being a mixed bacterial culture.

In our previous report, utilization of single sugars (glucose, galactose, mannose, xylose, arabinose, etc.) was studied using the same MFC configuration, and all sugars and sugar alcohols could be utilized efficiently (Catal *et al.,* 2008a; Catal *et al.*, 2008c). Previous reports showed that culturing microorganisms using a specific sugar inhibits/limits the transport systems of other sugars. For example, Nobre *et al.* (1999) reported that glucose-proton symport is subject to glucose repression, whereas the xylose-proton symport requires induction by the substrate in yeast (Nobre *et al.,* 1999), *C. shehatae* (Lucas *et al.,* 1986), *P. stipitis* (Kilian *et al.,*1993) (Nobre *et al.,* 1999). As glucose being the principal carbon source for most living organisms, glucose repression is a complex regulatory system, and many genes are involved as regulatory mechanisms for monitoring glucose availability (Carlson, 1987). The release of glucose from sucrose through the action of invertase in yeasts inhibits maltose metabolism (Araujo *et al.,* 2007). Sugar phosphotransferase system (PTS) catalyzes the concomitant transport and phosphorylation of several sugars in many bacteria such as *E.coli*, *Salmonella typhimurium* (Saier, 1977). Since several sugars are phosphorylated via the PTS, bacterial cells must have sugar specific proteins. For example, hexoses including glucose, mannose and fructose are similarly translocated by a distinct enzyme II complex in the membrane. The enzyme II^{glc} acts upon glucose, where as the enzyme II^{man} exhibits broad substrate specificity, acting on glucose, mannose, 2-deoxyglucose, glucosamine and fructose with the order of decreasing affinity (Saier, 1977). Although some bacteria choose some specific carbon sources, it appears that our mixed bacterial culture preferentially use the sugar mixtures. Mixed bacterial cultures have broader substrate utilization range than pure cultures which might require specific carbon sources for growth, therefore extensive work has to be completed in order to assess the substrate preference for each individual strain in the culture. Lendermann *et al.,* (1996) reported simultaneous utilization of mixtures of carbon sources (glucose, galactose, fructose) by using pure

E.coli culture. As an advantages of simultaneous utilization of sugar mixtures, they suggested that heterotrophic microorganisms can grow relatively fast even in the presence of low environmental substrate concentrations (Lendermann *et al.,* 1996). In batch cultures containing carbon sources, microorganisms frequently exhibit diauxic growth. The substrate supporting the highest growth rate is utilized first, whereas the consumption of the other substrate is repressed (Harder and Dijkhuizen, 1982). Although mixed cultures of microorganisms were used in our study, we observed preferential utilization of sugar mixtures at individual level.

Mohan *et al.* (2007) reported that accumulation of high concentration of VFAs lower voltage generation (Mohan *et al.,* 2007). Carboxylic acid production was observed in the initial stage of MFC operation and decreased afterwards. Although it was reported that higher VFA concentrations negatively affect electricity generation, our results show that VFAs may also play role in electricity generation. As acetic acid was observed in all experiments, acetogenic bacteria are common in our mixed culture. Our results might explain the reason of longer electricity generation when all sugars are utilized in MFCs, that bacteria might also utilize the produced carboxylic acids as carbon sources.

3.6 Effects of Operational Factors on Electricity Generation in Microbial Fuel Cells

3.6.1. Acclimation of electricity generating bacteria using sucrose at pH 7

MFC which was run with acetate as carbon source (2000 mg/L acetate, 100 mM buffer, pH 7) produced electricity. When acetate was replaced with sucrose (1100 mg/L), electricity production gradually reached to the maximum value of 0.31 mW. Following sucrose treatment required ~20 hrs before reaching the first maximum power (Fig. 3.48). The chamber was then refilled when the voltage decreased to 0.05 V. The time to reach the maximum voltage was shorter than that in the first batch of sucrose addition. It can be assumed that changing the carbon source may induce different metabolic reactions which require time for adaptation, or bacterial community may change. It was suggested that the different metabolic adaptations might be induced by the initial carbon source influenced some of redox reactions among the many metabolic reactions within the microorganism (Kim *et al.,* 2000).

Similarly, our result implies that bacteria in the MFC give a different reaction against sucrose due to the activation of different metabolic pathways.

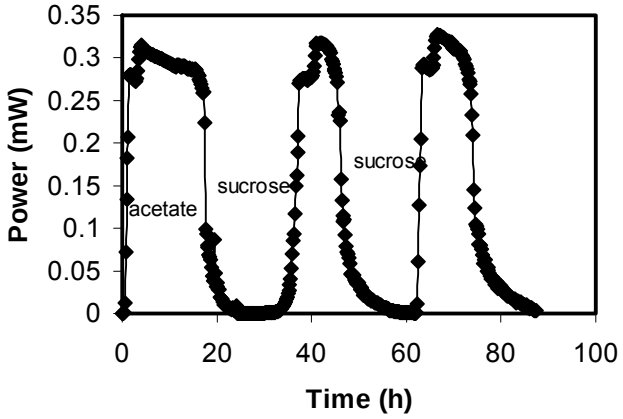

Figure 3.48: Maximum power production using 1100 mg/L sucrose solution which after replacement of acetate containing (2000 mg/L) medium during batch operation.

3.6.2 Effects of pH

The effects of pH changes on voltage production were illustrated in figure 3.49. Medium containing 1100 mg/L sucrose was treated in all experiments at pH varying between 5 and 9.5. Figure 3.49 illustrates the pH effect on electricity generation using 1k Ω resistor. Maximum voltage produced by the bacteria consuming sucrose at pH 7 was 0.49 V (1k Ω resistor), while voltage decreased upon increasing pH to 9.5. However, further increase of pH to 9.5 decreased the electricity production drastically while the steady state of power generation lasted longer. The length of stable electricity production period increased from 15 h at pH 7 to 50 h at pH 9.5. It was lower with that of pH 7, however a longer period of sustainable power generation was achived which was similar to that observed at pH 8.5. Our result indicates that even at the pH 9.5, the ability to generate electricity is not lost. In addition, experiments conducted with 1100 mg/L sucrose at a pH 5 indicates that electricity production was less than that at pH 7 and 9.5. Similar observations were reported previously that an increase of pH to 8.5 was associated with a decrease of power output (Rozendal *et al.,* 2006). It can be suggested that pH affects electricity production significantly, a longer duration of electricity production can be observed. The voltage was significantly lowered at pH 5, and electricity generation was not

stable. Electricity generation is affected negatively under pH 5.0 because the buffer capacity might be limited during operation. Fang and Liu (2002) reported that microbial community of a mixed bacterial culture changed when pH varied between 4.0-7.0 (Fang and Liu, 2002). It is possible that our bacterial community has greatly affected by pH and could have been changed resulting in different electricity generation profiles.

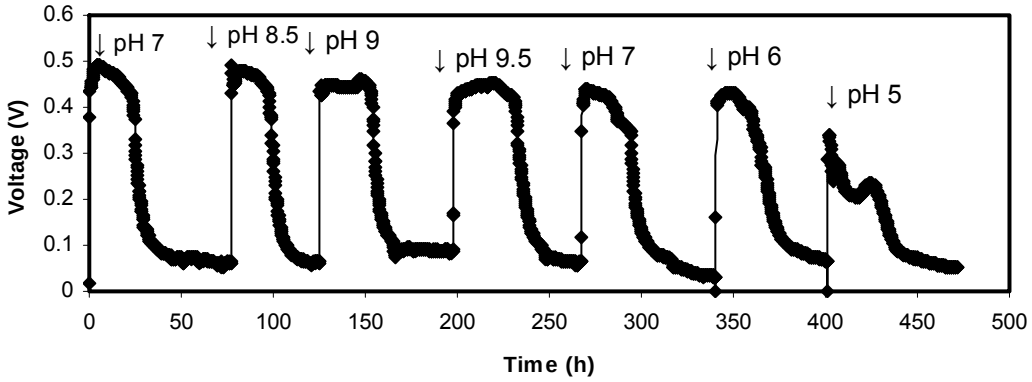

Figure 3.49: Voltage as a function of time at various pHs using a 1k Ω resistor.

Figure 3.50 illustrates the initial and final pH of the experiments. Although no significant pH changes were observed at the initial and final pH values of neutral pH treatment, a little decreasing in pH was observed in all pH experiments. However, at higher pH, this difference was bigger when compared to those at pH 7. This means that a decrease in pH can also be observed in MFC studies, which is a problem during batches. The level of pH reduction may depend on many factors such as substrate concentration, temperature and duration. Li and Fang (2006) reported that reduction of pH might be resulted from the production of fatty acids in anaerobic cultivation. It was also suggested that pH might affect the metabolic pathways in hydrogen production. For example, at pH 6, butyrate is predominant while at pH 6.5 acetate becomes predominant suggesting that the product profile affected by pH. In this study, although effluent solution was not analyzed for fatty acids, or other products mentioned above, it can be assumed that the formation of other fermentative products causing pH reduction could be expected.

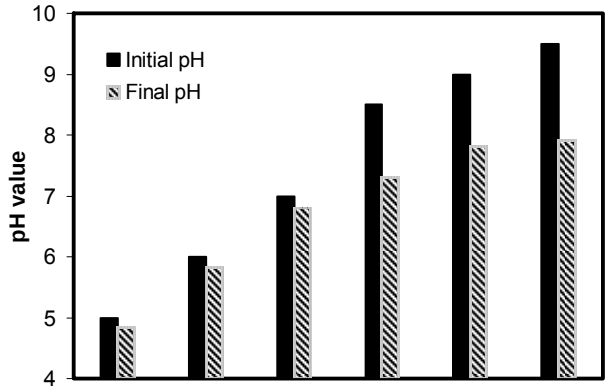

Figure 3.50: Initial and final pH throughout one batch. Values are based on the mean of duplicate experiments.

3.6.3 Effects of sucrose concentration

Treatment of increased concentrations of sucrose affected voltage production significantly. Gradually increasing the concentrations of substrate from 1100 to 11000 (mg L^{-1}) caused a gradual decrease of voltage production from 0.27 to 0.19 V using a 120 Ω fixed resistor (Fig. 3.51). It was previously been reported that the effect of substrate concentration on power output using 1k Ω resistance (Liu *et al.,* 2005). In our study, effect of substrate concentration is much clearer due to the use of lower resistance.

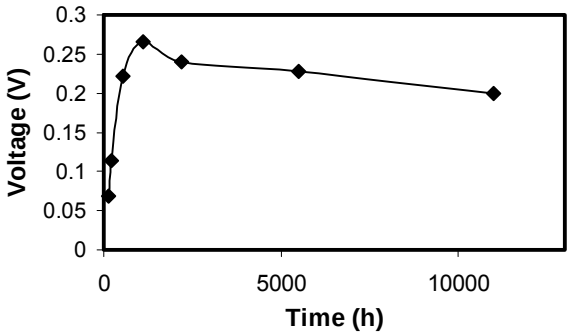

Figure 3.51: Voltage generation as a function of sucrose concentration.

3.6.4 Effect of anode surface area variation

The effects of anode surface area variation on power density was investigated on two different sizes (7 and 2 cm^2) in the MFC at pH 7 using sucrose (1100 mg L^{-1}) substrate. In both experiments, the power density was normalized to the anode surface surface area. For the larger anode (7 cm^2), power density did not increase above 1000 mW m^{-2}, whereas a higher power density was achived with smaller size (2 cm^2). Decreasing the anode size from 7 cm^2 to 2 cm^2 increased the power density from 898 mW/m^2 to 1966 mW/m^2 ,(~2.19 fold) (Fig. 3.52). The resistor was varied for each experiment so that the maximum power density could be produced. We observed that the power density was most strongly correlated to anode size. Thus, our result suggests that anode part of the MFC is not a limiting factor for electricity generation. However, a better evaluation of relative power densities reported here will be possible by comparison of power densities, on the basis of relative anode sizes. Previously, Liu *et al.* (2005) have reported a power density of 1330 mW/m^2 using a similar MFC system feeding with wastewater (Liu *et al.,* 2005). The power density of 29.2 W/m^3 was reported using sucrose as a substrate. However, the system was different from ours in configuration that an upflow type MFC was used in that study (He *et al.,* 2005).

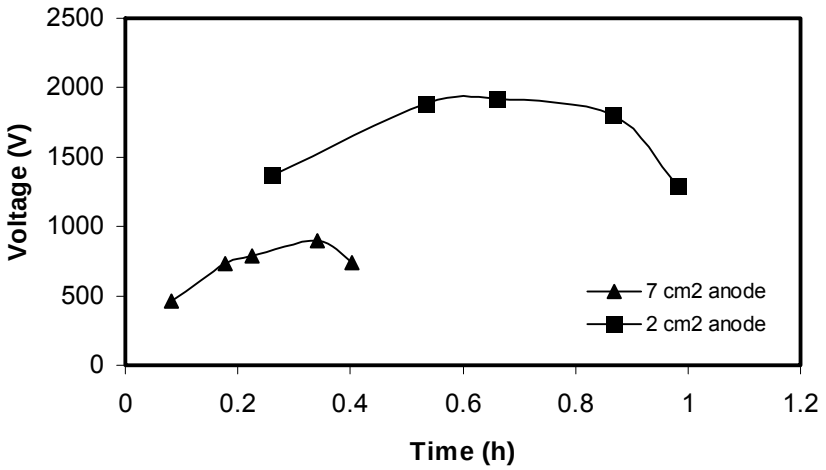

Figure 3.52: Power output (mW m^{-2}) of a sucrose-fed (1100 mg L^{-1}) MFC curve using 2 and 7 cm^2 anode surface areas.

Figure 3.53 shows the effects of pH on the performance of MFC ran in batch mode. Maximum power density of 1052 mW m^{-2} was achieved at pH 9. At pH 6, the power

density decreased significantly to 465 mW m^{-2}. These findings demonstrate that power density is strongly dependent on the pH. Similarly, Gil *et al.* (2003) showed similar results that pH may affect the current generation.

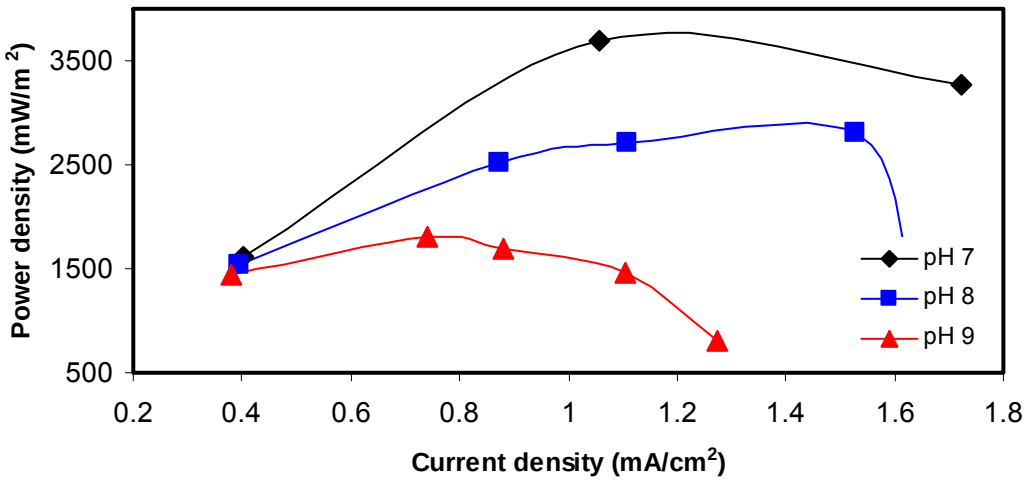

Figure 3.53: Effects of pH on power density.

3.6.5 Chemical oxygen demand removal

In approxymately 50 h, COD of sucrose decreased by 89.5%. A longer electricity production (coulombic efficiency) was maintained with acetate although Coulombic efficiency of sucrose was higher than that of acetate. This result indicates that bacterial mixed culture completely adapted to acetate could easily degrade it, and the coulombic efficiency is low. The utilization rate of sucrose (1100 mg L^{-1}) was around 98%. However, degradation of sucrose may produce some other secondary products such as acetate, butyrate, hydrogen and carbon dioxide, etc. (Fang *et al.*, 2002). Decreasing the anode area from 7 cm^2 to 2 cm^2 almost barely affected Coulombic efficiencies of larger and smaller anode were similar (30% and 29%, respectively).

Coulombic efficiencies showed a similar trend but the differences were more significant than power density. Coulombic efficiency of 19.57% was achived when sucrose completely oxidized at pH 6. Increasing the pH resulted in increased Coulombic efficiency from 19.57 to 36.6%. For example, at pH 9.5, Coulombic efficiency was 36.6 %. This result indicates that the Coulombic efficiency of the microbial fuel cell strongly depends on the initial pH of the medium. Low value of

Coulombic efficiency might be due to poor proton transfer at reduced proton concentration gradient across the membrane in two chamber MFC systems (Gil *et al.,* 2002). Here, although single chamber MFC was used, low Coulombic efficiency might be resulted from the effect of pH on proton gradient through anode and cathode. Cheng *et al.* (2006) reported 90-95% Coulombic efficiency using wastewater. The difference in Coulombic efficiencies caused by pH changes can be explained by the change in metabolic state of bacterial culture.

Increased loading of COD using sucrose causes a significant decrease in coulombic efficiency (He *et al.,* 2006). We observed a similar result that a treatment of increased sucrose concentration resulted in a decreased Coulombic efficiency. It can be postulated that increased loading rate of sucrose may result in some other products. The reason why increased concentration treatment does not result in a higher power output may be due to some other limiting factors related to the production of unwanted matters, electrode capacity and internal resistance (Min *et al.,* 2005). Increasing the ionic strength and decreasing the electrode spacing are suggested to decrease the internal resistance without changing the bulk solution pH (Liu *et al.,* 2005).

3.6.6. Biofilm formation on electrode surface

Anaerobic bacteria attached onto the anode surface forming a biofilm structure (Fig. 3.54). Graphite anode surface area and its porous structure are important in bacteria attachment. Furthermore, this porous structure may be beneficial for scale-up applications. Currently, the information about how bacteria attach to electrode surface remains unknown. However, Gregory *et al.* (2004) suggested that microorganisms need to be in direct contact with the electrode forming an apparent monolayer on the electrode surface especially when *Geobacter* species use electrodes as electron acceptor. It was shown that some species of bacteria such as *Shewanella* and *Geobacter* produce nanowires that are highly conductive. Therefore, bacteria that are not directly in contact with a surface achieve electron transfer to that surface (Gorby *et al.,* 2006). Neverthless, one reason might be the need of bacteria for attachment to a surface as in the immobilized form of the microorganims. Internal resistance of MFC can also be affected by research the biofilm because it is part of

the electrode (Aelterman *et al.,* 2006). SEM image of cathode appeared similar to the one obtained with the anode after biofilm formation (Fig.3.54).

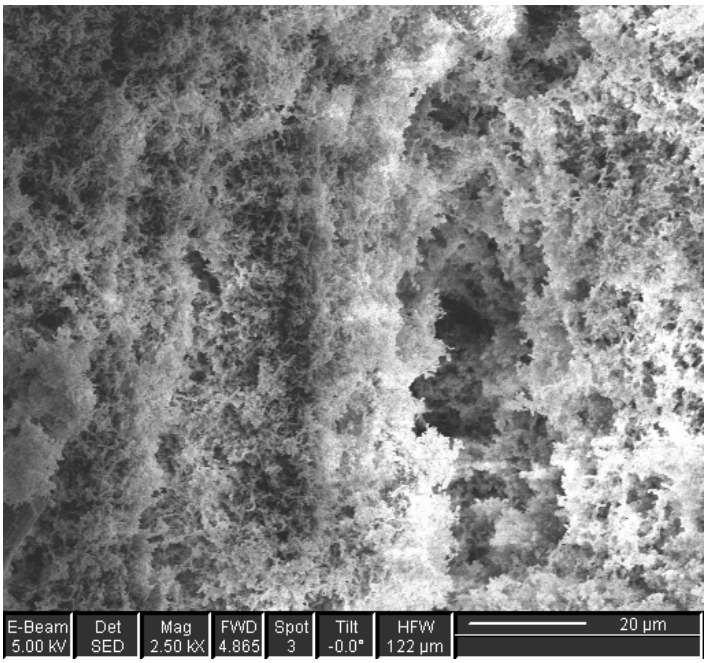

Figure 3.54: SEM image of anode with biofilm developed. Photograph by Y. Fan.

Wastewater treatment costs can be very high, and there is a great interest in MFC technology which links wastewater treatment with electricity generation (Oh *et al.,* 2005). Hydrogen can be produced instead of electricity in relatively high yields in MFCs. If a small voltage is applied to the circuit and oxygen is removed from the cathode, hydrogen gas can be evolved from the cathode (Logan and Regan, 2006). Researchers have reported hydrogen production from sugary wastewater (Ueno *et al.,* 1995; Liu *et al.,* 2003), and the role of sucrose concentrations and pH levels for hydrogen production was also reported (Ginkel *et al.,* 2001). Although electricity production from synthetic wastewater containing sucrose was reported (He *et al.,* 2005), the concentration and pH effects have not well understood.

Electricity production from artificial wastewater in MFC using sucrose as the electron donor was reported, and the maximum power density achieved was 170 mW m^{-2} (He *et al.,* 2005). Here we report the power density of 864 mW m^{-2} using sucrose. He *et al.* (2005) used hexacyanoferrate as a mediator which is a disadvantage for MFC due to the requirement of refreshing the solution during the operation. We used mediatorless type MFCs that makes this technology more useful

for long term applications. Moreover, our Coulombic efficiency was higher than that reported and COD removal was also higher.

3.6.7 Dynamics of Microbial community

Figure 3.55 illustrates the DGGE profiles of the 16S rDNA gene fragments amplified from DNA extracted from the biofilms on anodes of MFCs fed with various monosaccharides. Each band on the DGGE profile represents a specific species in the microbial community and staining intensity of a band represents the relative abundance of the corresponding microbial species. DGGE profiles clearly show that the microbial community changed with different substrates. Among the detectable bands in the DGGE profiles of the fours samples, only 4 bands were common in all the samples with band 3 demonstrating very highintensity in the samples with monosaccharides as carbon sources.

Figure 3.56 illustrates the DGGE profiles of 16S rDNA gene fragments amplified DNA extracted from the biofilms on anodes of MFCs fed with sorbitol (A), ribitol (B), galactitol (C), and mannitol (D). Each band on the DGGE profile represents a specific species in the microbial community and the staining intensity of a band represents the relative abundance of the corresponding microbial species. DGGE profiles in Figure 3.56 clearly show that the microbial community changed with different substrates. More bacterial species were presented in the anode biofilm of MFCs fed with hexitols, sorbitol (A) and mannitol (D), than that from the MFCs fed with pentitols, ribitol (B) and galactitol (C) (Fig. 3.56). However, power densities generated by sorbitol and mannitol were much lower than those by ribitol and galactital (Table 3.3), indicating that the fewer bacterial species in the latter samples may play a more important role in electron transfer from substrates to electrodes. Among the detectable bands in DGGE profiles of the four samples, only 4 bands were common in all the samples with band 3 demonstrating very high intensity in the samples with mannitol, ribitol and galactital as carbon sources.

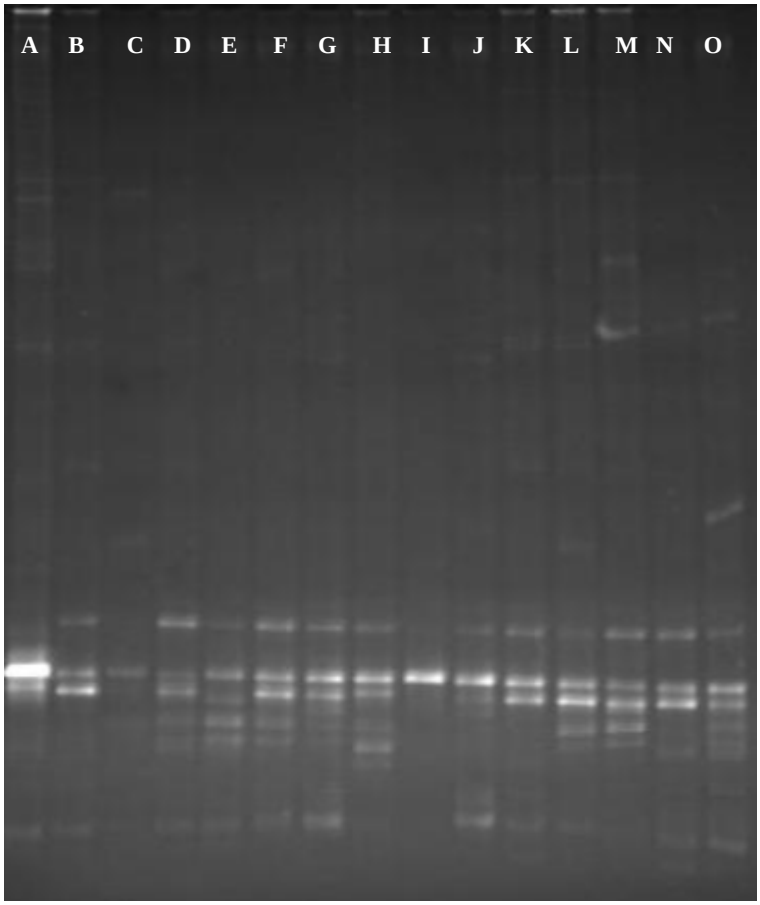

Figure 3.55: DGGE profiles of the 16S rDNA gene fragments amplified from DNA extracted from the biofilms on anodes of MFCs fed with various monosaccharides or disaccharide samples. Rhamnose (A), arabinose (B), galactose (C), fucose (D), sucrose (E), maltose (F), xylose (G), sorbose (H), maltose (I), gluconic acid (J), ribose (K), mannose (L), glucuronic acid (M), gluconic acid (N), galacturonic acid (O). Photo by S. Xu.

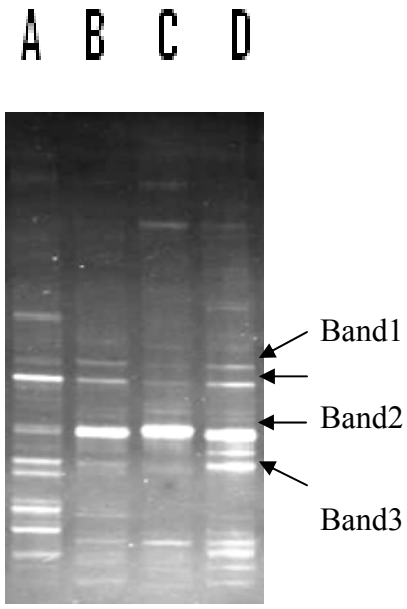

Figure 3.56: DGGE analysis of 16S rDNAs extracted from the MFCs using sorbitol (A), ribitol (B), galactitol (C), and mannitol (D) as carbon sources. Photo by S. Xu.

3.7 Electricity Generation Results from Pine Wood Flour Acidic Hydrolysate

Figure 3.57A shows the electricity generation from pine wood hydrolysate. As seen in the figure, electricity can be produced easily from the hydrolysate (light blue arrow). Figure 3.57B shows the direct electricity generation from pine wood flour (2.4 g/L) (Dark green arrow). For the first and second batches electricity could be produced from pine wood flour. In the following batches, fresh media without carbon source was added to understand if electricity continued be produced from remaining wood flour particles in MFCs. However, after addition of fresh media, electricity generation was not observed. Consequent addition of pwf could produce low electricity. At the end of the experiment, glucose was added to the MFC and electricity generation was recovered increasingly. The possible reason for decreased voltage after fresh media addition (red arrow) might be (1) either some inhibitor compounds were produced during degradation of pine wood flour (causing killing of bacteria), or (2) to keep the reactor contents unchanged (water evaporation, and conductivity problem through solution in MFC).

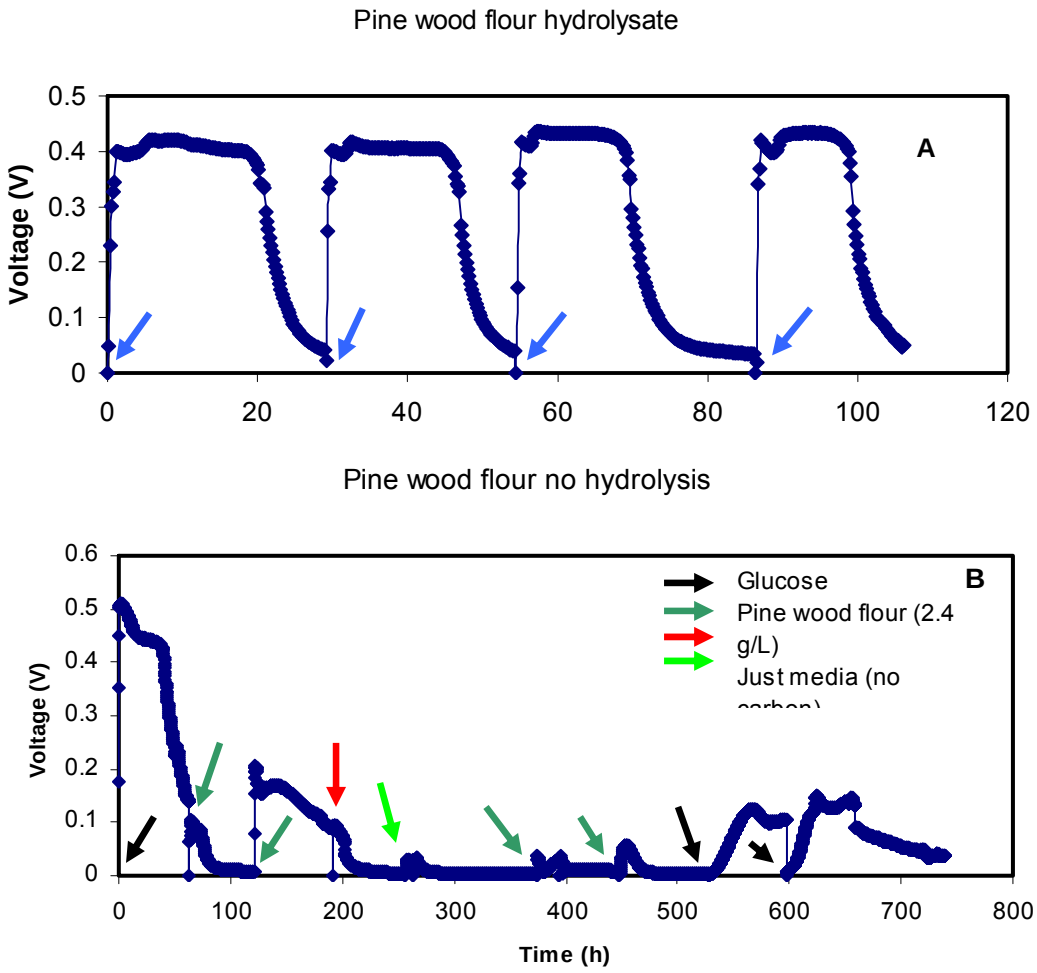

Figure 3.57: Electricity generation in MFCs from acid-hydrolysed (A) and non-hydrolysed pine wood flour.

4. CONCLUSIONS

Electricity was successfully generated from twelve monosaccharides (glucose, galactose, fructose, fucose, rhamnose, mannose, xylose, arabinose, ribose, galacturonic acid, glucuronic acid, gluconic acid), two disaccharides (maltose and cellobiose), and six polyalcohols (xyitol, arabitol, ribitol, galactitol, mannitol, sorbitol) using a mixed bacterial culture in single-chamber, air-cathode, mediator-less MFCs .The mixed bacterial culture enriched with and maintained in sodium acetate easily adapted to all carbon sources tested with the adaptation time ranging from 1 to 70 hours. The maximum power density ranged from 1262±5 mW m^{-2} to 2763±38 mW m^{-2} with glucuronic acid producing the highest power density and mannose producing the lowest one. Over 70 % of COD was removed for all carbon sources tested. The E_c ranged from 10 to 34 %. Our results indicated that all monosaccharides, disaccharides and polyalcohols in a hydrolysate from acid hydrolysis of lignocellulosic materials could be used for electricity generation.

Among the 2 furan derivative and 8 phenolic compounds tested in this study, electricity was produced only from 5-HMF with a voltage output much lower than that using glucose. All the other compounds tested were unable to directly produce electricity in MFCs in the absence of other electron donors. When glucose was used as the carbon source, electricity generation was not significantly affected by the addition of 5-HMF, *trans*-, 3,5-dimethoxy-4-hydroxy- cinnamic acids at a concentration up to 10 mM while syringaldeyhde, vanillin, *trans*-4-hydroxy-3-methoxy, and 4-hydroxy cinnamic acids inhibited voltage generation at concentrations above 5 mM. Electricity generation was severely inhibited by 2-furaldehyde, acetophenone and 3-4-dimethoxybenzyl alcohol at a concentration less than 0.2 mM.

If one were to consider using lignocellulosic material as substrates in MFCs the microorganisms encounter complex mixtures of sugars in the system. Regardless of the mixture composition, all sugars were utilized simultaneously to produce

electricity for 24h (~0.56V at 1k ohm resistance) in air cathode MFCs. However, the utilization rate was different when they tested in combination, and some sugars were preferred while others were utilized afterwards; and a significant amount of the added sugars were utilized in 2h. During the MFC operation carboxylic acid production was observed. Acetic acid and propionic acid (356±6 mg/L and 431±5 mg/L, respectively, using the combination of five sugars, at 2h) production was dominant and the production level varied depending on the sugar combination. Our results demonstrated that prefential utilization of sugar mixtures enables microorganisms to initiate electricity generation relatively fast even in the presence of low substrate concentrations.

DGGE of PCR-amplified 16S rRNA gene segments of the anode biofilms showed the influence of substrates (monosaccharides and polyalcohols) on the anode microbial populations. Our results demonstrated that air-cathode single-chamber MFCs exhibited a good electricity generation property from all substrates which showed a promising future application in this field using lignocellulosic biomass in an efficient way.

REFERENCES

Aelterman, P., Rabaey, K., Pham, H.T., Boon, N. and Verstraete, W., 2006. Continuous electricity generation at high voltages and currents using stacked microbial fuel cells, *Environ. Sci. Technol.,* **40**, 3388-3394.

Almeida, J.R.M., Modig, T., Petersson, A., Hähn-Hägerdal, B., Lidén, G. and Gorwa-Grauslund, M.F., 2007. Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae, J. Chem. Technol. Biotechnol.,* **82**, 340-349.

American Public Health Association, 1992. *American Water Works Association, Water Pollution Control Federation*, In Standart Methods for the Examination of Water and Wastewater. 18th ed.; American Public Health Association, Washington, DC.

Alves Araujo, C.A., Pacheco, A., Almeida, M.J., Spencer Martins, I., Leao, A. and Sousa, M.J., 2007. Sugar utilization patterns and respiro-fermentative metabolism in the baker's yeast *Torulaspora delbrueckii*, *Microbiology*, **153**, 898-904.

Bennetto, H. P., Stirling, J. L., Tanaka, K. and Vega C. A., 1983. Anodic Reaction in Microbial Fuel Cells, *Biotechnology and Bioengineering*, **25**, 559-568.

Bjerre, A.B., Olesen, A.B., Fernqvist, T., Ploger, A. and Schmidt, A.S., 1996. Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. *Biotechnology and Bioengineering*, **49**, 568-577.

Bond, D.R., Holmes, D.E., Tender, L.M. and Lovley, D.R., 2002. Electrode-reducing microorganisms that harvest energy from marine sediments, *Science.* **295**, 483-485.

Bond D.R. and Lovley, D.R., 2003. Electricity Production by *Geobacter sulfurreducens* Attached to Electrodes, *Appl. Environ. Microbiol.* **69**, 1548-1555.

Brigham, J.S., Adney, W.S. and Himmel, M.E., 1996. Hemicelluloses: diversity and applications. In: Wyman CE (ed) Handbook on bioethanol: production and utilization. pp.119-142, Taylor and Francis, Washington, DC.

Cantarella, M. Cantarella, L. Gallifuoco, Spera, A. and Alfani, F., 2004. Effect of inhibitors released during steam-explosion treatment of poplar

wood on subsequent enzymatic hydrolysis and SSF, *Biotechnol. Prog*., **20**, 200-206.

Carlson, M., 1987. Regulation of sugar utilization in Saccharomyces species, *Journal of Bacteriology,* **169**, 4873-4877.

Catal, T., Li, K., Bermek, H. and Liu, H., 2008a. Electricity Production from Twelve Monosaccharides Using Air Cathode Microbial Fuel Cells, *Journal of Power Sources,* **175**, 196-200.

Catal, T., Fan, Y., Li, K., Bermek, H. and Liu, H., 2008b. Effects of Furan Derivatives and Phenolic Compounds on Electricity Generation in Microbial Fuel Cells. *Journal of Power Sources*, **180**, 1: 162-166.

Catal, T., Xu, S., Li, K., Bermek, H., Liu, H., 2008c. Electricity Generation from polyalcohols in Microbial Fuel Cells, *Biosensors and Bioelectronics*, doi: 10.1016/j.bios/2008.07.015.

Chang, I.S., Jang, J.K., Gil, G.C., Kim, M., Kim, H.J., Cho, B.W. and Kim, B.H., 2004. Continuous determination of biochemical oxygen demand sensor using a microbial fuel cell type biosensor, *Biosens. Bioelectron.,* **19**, 607-613.

Chang, I.S., Moon, H., Jang, J.K. and Kim, B.H., 2005. Improvement of microbial fuel cell performance as a BOD sensor using respiratory inhibitors, *Biosens. Bioelectron*., **20**, 1856-1859.

Chaudhuri, S. K. and Lovley, D. R., 2003. ,Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nat. Biotechnol*., **21**, 1229-1232.

Chen, S.F., Mowery, R.A., Scarlata, C.J. and Chambliss, C.K., 2007. Compositional analysis of water-soluble materials in corn stover, *J. Agric. Food Chem.,* **55**, 5912-5918.

Cheng, S., Liu, H. and Logan, B.E., 2006. Increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing. *Environ. Sci. Technol*., **40**, 2426-2432.

Cheng, S., Liu, H. and Logan, B.E., 2006a. Increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing. *Environ. Sci. Technol*., **40**, 2426-32.

Cheng, S., Liu, H. and Logan, B.E., 2006b. Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells. *Environ. Sci. Technol*. **40**, 364-369.

Chheda, J.N., Huber, G.W. and Dumestic, J.A., 2007. *Angew. Chem. Int. Ed.* **46**, 2-22.

Clark, T.A. and Mackie, K.L., 1984. Fermentation inhibitors in wood hydrolysates derived from the softwood *Pinus radiate*, *Appl. Biochem. Biotechnol.,* **24**/251.

Cohen, B., 1931. The Bacterial Culture as an Electrical Half-Cell, Journal of *Bacteriology*, **21**, 18-19.

Davis, F. and Higson, S.P.J., 2007. Biofuel cells—Recent advances and applications, *Biosens Bioelectron.,* **22**, 1224-1235.

Dominguez, A., Rivela, I., Couto, S.R. and Sanromán, M.A., 2001. Design of a new rotating drum bioreactor for ligninolytic enzyme production by *Phanerochaete chrysosporium* grown on an inert support, *Process Biochemistry,* **37**, 549–554.

Dumas, C., Basseguy, R. and Bergel, A., 2008. Electrochemical activity of Geobacter sulfurreducens biofilms on stainless steel anodes, *Electrochimica Acta*, **53**, 5235-5241.

Ediger, V.S. and Akar, E., 2007. ARIMA forecasting of primary energy demand by fuel in Turkey, *Energy policy*, **35**, 1701-1708.

Fan, Y., Hu, H., and Liu, H. 2007. Enhanced Coulombic efficiency and power density of air-cathode microbial fuel cells with an improved cell configuration, *Journal of Power Sources*, **171,** 348-354.

Fang, H.H.P. and Liu, H., 2002. Effect of pH on hydrogen production from glucose by a mixed culture, *Bioresource Technology,* **82**, 87-93.

Fang, H.H.P., Liu, H. and Zhang, T., 2002a. Characterization of a hydrogen-producing granular sludge. *Biotechnology and Bioengineering*, **78**, 44-52.

Fang, H.H.P., Liu, H. and Zhang, T., 2005b. Phototrophic hydrogen production from acetate and butyrate in wastewater, *International Journal of Hydrogen Energy*, 30, 785-793.

Fenice, M., Giovannozzi, S.G., Federici, F. and D'Annibale, A., 2003. Submerged and solid-state production of laccase and Mn-peroxidase by *Panus tigrinus* on olive mill wastewater-based media, *J. Biotechnol*. **100,** 77–85.

Freguia, S., Rabaey, K., Yuan, Z. and Keller, J., 2007. Non-catalyzed cathodic oxygen reduction at graphite granules in microbial fuel cells *Electrochimica Acta*, **53**, 598-603.

Gil, G.C., Chang, I.S., Kim, B.H., Kim, M., Jang, J.K., Park, H.S. and Kim, H.J., 2002. Operational parameters affecting the performance of a mediator-less microbial fuel cell, *Biosensors and Bioelectronics*, **18**, 327-334.

Ginkel, S.V., Sung, S and Li, L., 2001. Role of initial sucrose and pH levels on natural, hydrogen-producing, anaerobe germination. *Proceedings of the 2001 DOE Hydrogen Program Review*. NREL/CP-570-30535.

Gorby, Y.A., Yanina, S., McLean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., Beeveridge, T.J., Chang, I.S., Kim, B.H., Kim, K.S., Culley, D.E., Reed, S.B., Romine, M.F., Saffarini, D.A., Hill, E.A., Shi, L., Elias, D.A., Kennedy, D.W., Pinchkuk, G., Watanabe, K., Ishii, S., Logan, B., Nealson, K.H. and Fredrickson, J.K., 2006. Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms, *PNAS.*, **103**, 11358-11363.

Gregory, K.B., Bond, D.R. and Lovley, D.R., 2004. Graphite electrodes as electron donors for anaerobic respiration. *Environmetal Microbiology,* **6**, 596-604.

HaoYu, E., Cheng, S., Scott, K. and Logan, B., 2007. Microbial fuel cell performance with non-Pt cathode catalysts, *Journal of Power Sources*, **171**, 275-281.

Harder, W. and Dijkhuizen, L., 1982. Strategies of mixed substrate utilization in microorganisms, *Philos. Trans. R. Soc. Lond. B Biol. Sci.,* **297**, 459-480.

He, Z., Wagner, N., Minteer, S.D. and Angenent, L.T., 2006. An upflow microbial fuel cell with an interior cathode: assessment of the internal resistance by impedance spectroscopy, *Environ. Sci. Technol*., **40**, 5212-5217.

He, Z., Shelley, M.D., Largus, A.T., 2005. Electricity generation from artificial wastewater using an upflow microbial fuel cell, *Environ. Sci. Technol.*, **39**, 5262-5267.

Heijne, A., Hamelers, H.V.M., Saakes, M. **and Buisman, C.J.N.,** 2008. Performance of non-porous graphite and titanium-based anodes in microbial fuel cells, *Electrochimica Acta*, **53**, 5697-5703.

Heilmann, J. and Logan, B.E., 2006. Production of electricity from proteins using a single chamber microbial fuel cell, *Water Environ. Res.,* **78**,1716-1721.

Hinman, N.D., Wright, J.D., Hoagland, W. and Wyman, C.E., 1989. Xylose fermentation. An economic analysis. *Applied Biochemistry and Biotechnology*. **20-21,** 391-401.

Karube, I., Matsunga, T., Mitsuda, S., Suzuki, S., 1977. Microbial. electrode BOD sensors, *Biotechnol. Bioeng.,* **19**, 1535-1547.

Kermanshahi pour, A., Karamanev, D. and Margaritis, A., 2005. Biodegradation of petroleum hydrocarbons in an immobilized cell airlift bioreactor, *Water Research*, **39**, 3704-3714.

Kerstetter, J.D. and Lyons, J.K., 2001. *Wheat straw for ethanol production in Washington: a resource, technical, and economic assessment.* Washington State University Cooperative Extension Energy Program.

Kilian, S.G., Prior, B.A. and du Preez, J.C., 1993. The kinetics and regulation of D-xylose transport in *Candida utilis*, *World J. Microbiol. Biotechnol*., **9**, 357-360.

Kim, H.J., Hyun, M.S., Chang, I.S. and Kim, B.H., 1999. A microbial fuel cell type biosensor using a metal-reducing bacterium, *Shewanella putrefaciens*., *J. Microbiol. Biotechnol.,* **9**, 265-267.

Kim, N., Choi, Y., Jung, S. And Kim, S., 2000. Effect of initial carbon sources on the performance of microbial fuel cells containing *Proteus vulgaris, Biotechnol. Bioeng*., **70**, 109-14.

Kim, B.H., Chang, I.S., Gil, G.C., Park, H.S. and Kim, H.J., 2003. Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell, *Biotechnology Letters* **25**, 541-545.

Kim, G.T., Webster, G., Wimpenny, J.W.K., Kim, B.H., Kim, H.J. and Weihtman, A.J., 2006. Bacterial community structure, compartmentalization and activity in a microbial fuel cell, *Journal of Applied Microbiology*, 101, 698-710.

Kim, J.R., Jung, S.H., Regan, J.M. and Logan, B.E., 2007. Electricity generation and microbial community analysis of alcohol powered microbial fuel cells, *Bioresour. Technol.*, **98**, 2568-2577.

Klinke, H.B. Ahring, B.K., Schmidt, A.S., and Thomsen, A.B., 2002. Characterization of degradation products from alkaline wet oxidation of wheat straw, *Bioresource Technol.,* **82**, 15-26.

Kumlanghan, A., Liu, J., Thavarungkul, P., Kanatharana, P., Mattiasson, B., 2007. Microbial fuel cell-based biosensor for fast analysis of biodegradable organic matter, *Biosensors and Bioelectronics,* **22**, 2939-2944.

Kuyper, M., Toirkens, M.J., Diderich, J.A., Winkler, A.A., van Dijken, J.P., and Pronk, J.T., 2005. Evolutionary engineering of mixed-sugar utilization by a xylose-fermentig *Saccharomyces cerevisiae* stratin, *Fems Yeast Research*, **5**, 925-934.

Larsson, S. Reimann, A. Nilvebrant and N. Johnsson, L.J., 1999. Comparison of different methods for the detoxification of lignocellulose hydrolysates of spruce, *Appl. Biochem. Biotechnol.,* **77**, 91-103.

Larsson, S. Cassland, P., and Jonsson, L.J., 2001. Development of a *Saccharomyces cerevisiae* Strain with Enhanced Resistance to Phenolic Fermentation Inhibitors in Lignocellulose Hydrolysates by Heterologous Expression of Laccase, *Appl. Environ. Microbiol.,* **67**, 1163-1170.

Lendermann, U., Snozzi, M. and Egli, T., 1996. Kinetics of simultaneous utilization of sugar mixtures by *Escherichia coli* in continuous culture, *Applied and Environmental Microbiology*, **62**, 1493-1499.

Liu, Z.D. and Li, H.R., 2007**.** Effects of bio- and abio-factors on electricity production in a mediatorless microbial fuel cell, *Biochemical Engineering Journal*, **36**, 209-214.

Liu, H. and Logan, B.E., 2004. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. *Environ. Sci. Technol*., **38**, 4040-4046.

Liu, H., Zhang, T. and Fang, H.H.P., 2003. Thermophilic H_2 production from a cellulose-containing wastewater, *Biotechnology Letters*, 25, 365-369.

Liu, H., Ramnarayanan, R. and Logan, B.E., 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell, *Environ. Sci. Technol.,* **38**, 2281-2285.

Liu, H., Cheng, S. and Logan, B.E., 2005. Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration, *Environ. Sci. Technol*., **39**, 658-662.

Liu, H., Cheng, S., Huang, L. and Logan. B.E., 2008. Scale-up of membrane-free single-chamber microbial fuel cells, *J. Power Sources*, **179**, 274-279.

Logan, B.E. and Regan, J.M., 2006. Electricity-producing bacterial communities in microbial fuel cells. *Trends in Microbiology.* **14**, 512-518.

Logan, B.E., Murano, C., Scott, K., Gray, N.D. and Head, I.M., 2005. Electricity generation from cysteine in a microbial fuel cell. *Wat. Res.,* **39,** 942-952.

Logan, B.E., Hamelers, B., Rozendal, R., Schroder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W. and Rabaey, K., 2006. Microbial fuel cells: methodology and technology, *Environ. Sci. Technol*., **40**, 5181-5192.

London, J. and Chace, N.M., 1977. New Pathway for the Metabolism of Pentitols *Proc. Natl. Acad. Sci. USA Biochemistry,* **74**, 4296-4300.

Lovley, D. R., 2006a.Microbial fuel cells: novel microbial physiologies and engineering approaches, *Curr. Opin. Biotechnol.,* **17**, 327-332.

Lovley, D.R., 2006b. Bug juice: harvesting electricity with microorganisms. *Nat. Rev. Microbiol.*, **4**, 497-508.

Lovley, D.R. and Phillips, E.J.P., 1988. Novel mode of microbial energy-metabolism-organic carbon oxidation coupled to dissimilatory reduction of iron or manganese, *Appl. Environ. Microbiol.,* **54**, 1472-1480.

Lucas, C. and van Uden, N., 1986. Transport of hemicellulose monomers in the xylose-fermenting yeast *Candida shehatae*, *Appl. Micobiol. Biotechnol*., **23**, 491-495.

Martin, C. and Jonsson, L.J., 2003. Comparison of the resistance of industrial and laboratory strains of *Saccharomyces* and *Zygosaccharomyces* to lignocellulosic-derived fermentation inhibitors, *Enzyme Microbial Technol*., **32**, 386-395.

Magnuson, T. S., Isoyama, N., Hodges-Myerson, A.L., Davidson, G., Maroney, M.J., Geesey, G.G. and Lovley, D.R., 2001. Isolation, characterization, and gene sequence analysis of a membrane associated 89 kDa Fe(III) reducing cytochrome c from *Geobacter sulfurreducens*, *Biochem. J.*, **359**, 147–152.

McMillan, J.D., 1994. Pre-treatment of lignocellulosic biomass. In: Himmel, M.E., Baker, J.O., Overend, R.P., (eds), Enzymatic conversion of biomass for fuels production. *ACS Symp Ser*, 411-437.

Min, B., Kim, J.R., Oh, S., Regan, J.M. and Logan, B., 2005. Electricity generation from swine wastewater using microbial fuel cells, Water Research, **39**, 4961-4968.

Min, B., Cheng, S. and Logan B.E., 2005a. Electricity generation using membrane and salt bridge microbial fuel cells, *Water Research*, **39**, 1675-1686.

Min, B., Kim, J.R. Oh, S. Regan, J.M. and Logan, B., 2005b. Electricity generation from animal wastewater using microbial fuel cells, *Wat. Res*., **39**, 4961-4968.

Mokrasch, L.C., 1954. Analysis of hexose phosphates and sugar mixtures with the anthrone reagent, *J. Bıol. Chem.,* **208**, 55-59.

Mohan, S.V., Raghavulu, S.V., Srikanth, S. and Sarma, P.N., 2007. Bioelectricity production by mediatorless microbial fuel cell under acidophilic condition using wastewater as substrate: influence of substrate loading rate, *Current Science*, **92**, 1720-1726.

Mohan, V.S., Veer Raghavulu, S. and Sarma, P.N., 2008a. Influence of anodic biofilm growth on bioelectricity production in single chambered mediatorless microbial fuel cell using mixed anaerobic consortia, *Biosensors and Bioelectronics,* (available online 21 March 2008).

Mohan, S.V., Mohanakrishna, G., Srikanth, S. and Sarma, P.N., 2008b. Harnessing of bioelectricity in microbial fuel cell (MFC) employing aerated cathode through anaerobic treatment of chemical wastewater using selectively enriched hydrogen producing mixed consortia, *Fuel*, **87**, 2667-2676.

Mohan, S.V., Saravanan, R., Raghavulu, S.V., Mohanakrishna, G. and Sarma, P.N., 2008c. Bioelectricity production from wastewater treatment in dual chambered microbial fuel cell (MFC) using selectively enriched mixed microflora: Effect of catholyte, *Bioresource Technology*, **99**, 596-603.

Morris, J.M., Jin, S., Wang, J., Zhu, C. and Urynowicz, M.A., 2007. Lead dioxide as an alternative catalyst to platinum in microbial fuel cells, *Electrochemistry Communications*, **9**,1730-1734.

Muyzer, G., Dewaal, E.C. and Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, *Appl. Environ. Microbiol.,* **59**, 695–700.

Nilvebrant, N.O., Persson, P., Reimann, A., De Sousa, F., Gorton, L. and Jonsson, L.J., 2003. Limits for alkaline detoxification of dilute-acid lignocellulose hydrolysates. *Appl. Biochem. Biotechnol.,* **105-108,** 615-628.

Nobre, A., Lucas, C. and Leao, C., 1999. Transport and utilization of hexoses and pentoses in the halotolerant yeast *Debaryomyces hansenii*, *Applied and Environmental Microbiology,* **65**, 3594-3698.

Oh, S., Min, B. and Logan B.E., 2004. Cathode performance as a factor in electricity generation in microbial fuel cells, *Environ. Sci. Technol*., **38**, 4900-4904.

Oh, S., Logan and B.E., 2005. Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies, *Water Research*, **39**, 4673-4682.

Ohgren, K., Rudolf, A., Galbe, M. and Zacchi, G., 2006. Fuel ethanol production from steam-pretreated corn stover using SSF at higher dry matter content, *Biomass Bioenergy*, **30**, 863-869.

Oliva, J.M., Saez, F., Ballesteros, I., Gonzalez, A., Negro, M.J., Manzanares, P. and Ballesteros, M., 2003. Effect of lignocellulosic degradation compounds from steam explosion pretreatment on ethanol fermentation by thermotolerant yeast *Kluyveromyces marxianus*, *Appl. Biochem. Biotechnol*. **105-108**, 141-153.

Palmqvist, E. and Hahn-Hagerdal, B., 2000. Fermentation of lignocellulosic hydrolysates: I: inhibition and detoxification, *Bioresource Technol.,* **74**, 17-24.

Pan, X. and Sano, Y., 2005, Fractionation of wheat straw by atmospheric acetic acid process. *Bioresource Technology*, **96**, 1256-1263.

Petrus, L. and Noordermeer, M.A., 2006, Biomass to biofuels, a chemical perspective, *Green Chemistry*, **8**, 861-867.

Picioreanu, C., Head, I.M., Katuri, K.P., van Loosdrecht, M.C.M. and Scott, K., 2007. A computational model for biofilm-based microbial fuel cells, *Water Research*, **41**, 2921-2940.

Potter, M. C., 1912. Electrical effects accompanying the decomposition of organic compounds. Royal *Society (Formerly Proceedings of the Royal Society) B*, **84**, 290-276.

Prasad, D., Arun, S., Murugesan, M., Padmanaban, S., Satyanarayanan, R.S., Berchmans S. and Yegnaraman, V., 2007. Direct electron transfer with yeast cells and construction of a mediatorless microbial fuel cell, *Biosens. Bioelectron.,* **22**, 2604-2610.

Qiao, Y., Li, C.M., Bao, S.J. and Bao Q.L., 2007. Carbon nanotube/polyaniline composite as anode material for microbial fuel cells, *Journal of Power Sources*, **170**, 79-84.

Rabaey, K. and Verstraete, W., 2005. Microbial fuel cells: sustainable core technology, *Trends Biotechnol*.. **23**, 291-298.

Rabaey, K., Boon, N., Siciliano, S.D., Verhaege, M. and Verstraete, W., 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer, *Appl. Environ. Microbiol.,* **70**, 5373-5382.

Rabaey, K., Ossieur, W., Verhaege, M. and Verstraete, W., 2005. Continuous microbial fuel cells covert carbohydrates to electricity, *Water Sci. Technol*., **52**, 515-23.

Rabaey, K., Sompel, K.V.D., Maignien, L., Boon, N., Aelterman, P., Clauwaert, P., Schamphelaire, L.D., Pham, H.T., Vermeulen, J., Verhaege, M., Lens, P. and Verstraete, W., 2006. Microbial fuel cells for sulfide removal, *Environ. Sci. Technol.,* **40**, 5218-5224.

Ragauskas, A.J., Nagy, M., Kim, D.H., Eckert, C.A., Hallett, J.P. and Liotta, C.L., 2006. From wood to fuels: integrating biofuels and pulp production. *Industrial biotechnology*, **2**, 55-65.

Rao, R.S. Jyothi, C.P. Prakasham, R.S. Sarma, P.N. and Rao, L.V., 2006. Xylitol production from corn fiber and sugarcane bagasse hydrolysates by *Candida tropicalis*. Bioresource Technol. 97 (2006) 1974.

Reimers, C.E., Girguis, P., Stecher III, H.A., Tender, L.M., Ryckelynck, N. and Whaling, P., 2006. Microbial fuel cell energy from an ocean cold seep, *Geobiology*. **4**, 123-136.

Ren, Z., Ward, T.E., and Regan, J.M., 2007. Electricity production from cellulose in a microbial fuel cell using a defined binary culture, *Environ. Sci. Technol.,* **41**, 4781-4786.

Rezaei, F., Richard, T.M., Brennan, R.A. and Logan, B.E., 2007. Substrate-enhanced microbial fuel cells for improved remote power generation from sediment-based systems, *Environ. Sci. Technol.,* **41**, 4053-4058.

Rosenbaum, M., Zhao, F., Quaas, M., Wulff, H., Schröder, U. and Scholz, F., 2007. Evaluation of catalytic properties of tungsten carbide for the

anode of microbial fuel cells, *Applied Catalysis B: Environmental*, **74**, 261-269.

Rozendal, R.A., Hamelers, H.V.M. and Buisman, C.J.N., 2006. Effects of membrane cation transport on pH and microbial fuel cell performance, *Environ. Sci. Technol.,* **40**, 5206-5211.

Saier, M.H., 1997. Bacterial phosphoenolpyruvate: sugar phosphotransferase systems: structural, functional and evolutionary interrelationships, *Bacteriological reviews*, **41**, 856-871.

Scott, K., Rimbu, G.A., Katuri, K.P., Prasad, K.K. and Head, I.M., 2007. Application of Modified Carbon Anodes in Microbial Fuel Cells, *Process Safety and Environmental Protection*, **85**, 481-488.

Stams, A.J.M., Bok, F.A.M.D., Plugge, C.M., Eekert, M.H.A.V., Dolfing, J., and Schraa, G., 2006. Exocellular electron transfer in anaerobic microbial communities, *Environmental Microbiology,* **8**, 371-382.

Sutherland, I.W., 2001. The biofilm matrix-an immobilized but dynamic microbial environment, *Trends Microbiol.,* **9**, 222-227.

Taherzadeh, M., Gustafsson, L., Niklasson, C., and Liden, G., 1999. Conversion of furfural in aerobic and anaerobic batch fermentation of glucose by *Saccharomyces cerevisiae. J. Biosci. Bioeng.,* **87**, 169-174.

Ueno, Y., Kawai, T., Sato, S., Otsuka, S. and Morimoto, M., 1995. Biological production of hydrogen from cellulose by natural anaerobic microflora, *J. Ferment. Technol.,* **79**, 395-397.

You, S., Zhao, Q., Zhang, J., Jiang, J. and Zhao S., 2006. A microbial fuel cell using permanganate as the cathodic electron acceptor, *Journal of Power Sources*, **162**, 1409-1415.

You, S., Zhao, Q., Zhang, J., Jiang, J., Wan, C., Du, M. and Zhao, S., 2007. A graphite-granule membrane-less tubular air-cathode microbial fuel cell for power generation under continuously operational conditions, *Journal of Power Sources*, **173**, 172-177.

You, S., Zhao, Q., Zhang, J., Liu, H., Jiang, J. and Zhao, S., 2008. Increased sustainable electricity generation in up-flow air-cathode microbial fuel cells, *Biosens. Bioelectron*., **23**, 1157-1160.

Yumurtaci, Z. and Asmaz, E., 2004. Electric Energy Demand of Turkey for the Year 2050, *Energy Sources*, **26**, 1157-1164.

Wiselogel, A., Tyson, J. and Johnsson, D., 1996. Biomass feedstock resources and composition. In: Wyman CE (ed) Handbook on bioethanol: production and utilization. , pp.105-118, Taylor and Francis, Washington, DC.

Zhang, T., Cui, C., Chen, S., Yang, H. and Shen, P., 2008. The direct electrocatalysis of Escherichia coli through electroactivated excretion in microbial fuel cell, *Electrochem. Comm.*, **10**, 293-297.

Zuo, Y., Maness, P.C. and Logan, B.E., 2006. Electricity production from steam-exploded corn stover biomass, *Energy&Fuels***, 20**, 1716-1721.

Zuo, Y., Xing, D., Regan, J.M. and Logan, B.E., 2008. Isolation of the exoelectrogenic bacterium Ochrobactrum anthropi YZ-1 by using a U-tube microbial fuel cell, *Appl. Environ. Microbiol*., **74**, 3130-3137.

RESUME

Tunc Catal was born in Germany (1980). He attened primary school at Bostanci Ilkokulu, then started Uskudar Fen Lisesi, and was graduated from Bilfen Lisesi (Istanbul) (1997). At the same year, he started the undergraduate program of Biology at Istanbul University, and was graduated in honor list (2001). He continued master program at the same department, and graduated in 2004. He has been pursuing his PhD research at Advanced technologies in engineering, Molecular Biology-Genetics and Biotechnology graduate program (ITU) and at the Department of Wood Science and Engineering and Department of Biological and Ecological Engineering at Oregon State University (USA) since 2004.