工學碩士 學位論文

Factors influencing on the performance of MFC (microbial fuel cell) system for wastewater treatment

하폐수처리를 위한미생물 연료전지공정의 성능에 대한 영향인자

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ABSTRACT

The Microbial fuel cell (MFC) is a device that converts chemical energy to electrical energy by the catalytic reaction of microorganisms. The present study was to identify the basic characteristics of a MFC, for example, kinds of microorganism (e.g suspended microorganism, attached microorganism), environmetal condition (e.g. starvation), redox mediator (electron shutle) and microorganism sources (types of inoculum), which relate to microbial activity, internal resistance of MFC system and overpotential loss during electricity generation in MFC. Batch mode likely H-shape (dual chambered batch) was used for all experiments in this study.

For improvement of electricity generation, this study examined some major factors influencing MFC performance such as the role of suspended (VSS) and/or attached microorganisms (biofilm) in the electron production, influence of redox mediators (e.g. Fe^{3+}/Mn^{4+} ions) on electron transfer in anode compartment and electric potential of different inoculums (e.g. activated sludge, anaerobic sludge and heat-treated sludge). In addition to, potential of hydrogen production in anode compartment using various bacterial sources were also observed.

To determine the effect of VSS and biofilm, power output under various environmental conditions (e.g. starvation, substrate concentration) was analized. The perusal of the results showed that internal resistance of the MFC system was reduced by the control of VSS concentration. Further, power output could be increased to higher value in the presence of biofilm on the surface of anode electrode than the electrode without attached to microorganism. Under starved condition, the longer operation the shorter steady stage was maintained. In this study, however, starvation gave a negligible effect on electricity generation of MFC.

Starvation effect on power output by biofilm doesn’t get higher yield than no starvation.

Electron transfer from bacteria to anode electrode was significantly enhanced by the presence of ferric and manganese ions. Maximum current of 2.06-1.92 mA and power density of 69.02-61.84 mW/m^2 were obtained from Fe-MFC and Mn-MFC, respectively. The coulbomb efficiency of Fe-MFC and Mn-MFC were enhanced up to 2-3 folds comparing to control MFC.

Maximum potential of hydrogen production and maximum rate were estimated by simulating a curve of cumulative hydrogen production using Modified Gompertz equation. According to Modified Gompertz equation, 1) MFC inoculated with anaerobic sludge gave highest yield of hydrogen production (46.91%) and the best performance coulomb efficiency of 66.36%, 2) MFC inoculated with heat treated sludge showed 17.80 % yield of hydrogen production and the coulomb efficiency of 47.20%, 3) MFC inoculated by activated sludge achieved only yield of 12.65% hydrogen production and 46.80% of coulomb efficiency.

The observed results are significant in two major respects: 1) improvement of electron transfer by medox mediator (using ferric and manganese ions) and 2) capability of MFC system produces hydrogen gas in anode compartment. Based on this work, the findings have important implications for developing a continuous MFC reactor in further study.

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LIST OF ABBREVIATIONS

ADP: Adenosine diphosphate

AnS: Anaerobic anaerobic sludge

AS: Activated sludge

ATP: Adenosine triphosphate

BOD: Biochemical oxygen demand

CE: Coulomb efficiency

COD: Chemical oxygen demand

DW: Deionized water

F/M: Food organism ratio

HRT: Hydrolic retention time

HTS: Heat-treated sludge

MFC: Microbial fuel cell

ML-MFC: Membraneless microbial fuel cell

MLSS: Mixed liquor suspended solids

MRB: Metal reducing bacteria

NADH/NAD^+: Nicotinamide adenine dinucleotide, oxidizing form (NAD^+) and its reducing form (NADH)

OCV: Open circuit voltage

OLR: Organic loading rate

ORP: Oxidation-reduction potential

PBS: Phosphate buffer solution

PEM: Proton exchange membrane

PVC: Polyvinyl Chloride

SCMFC: Single chamber microbial fuel cell

SRT: Sludge retention time

SS: suspended solid

SVI: Sludge volume index

VFA: Volatile fatty acid

WWTP: Wastewater treatment plant

CHAPTER 1
INTRODUCTION

1.1 Renewable energy technologies

Global industrial growth increases the demand for fossil fuels and energy. It is important to find an alternative energy source before the deleption of world's fossil fuels. Furthermore, developments increases a need of fuel consumption accompanies with a growth of environmental concern about global warming by CO_2 emissions. These problems are provided the stimulus to find a way to improve energy conversion efficiency. Since that time, clean energy technologies have considered an important alternative source for replacing fossil fuel. We have been harnessing energy comes from water falling and wind for hundreds of years. Hydropower, wind energy, solar power, biomass power, ocean energy and fuel cells are important. Solar and ocean's energies have been developed currently. Solar technologies convert the sun's energy to heat, light, hot water, electricity and cooling for homes, businesses, and industrial purposes. Current technologies include photovoltaics, concentrating solar, solar hot water, and more. It is free and does not pollute like wind energy. In addition to, conventional renewable energy source, ocean energy such as wave energy, tidal energy and ocean thermal energy conversion is a technology that uses their energy to spin turbines or converts solar radiation to electric power. Recently, an interest in fuel cell technology has expanded and it's a chemical energy to generate electricity from hydrogen, methane and methanol gas. Microbial fuel cells (MFC) are capable of converting chemical energy into electrical energy. In this respect, electricity can be produced from the degradation of organic matter by microorganisms in a MFC, which is

usually made up of two chambers; one anaerobic and one aerobic. In the anaerobic chamber the organic material is oxidized by the microbes and the lost electrons are transferred to the anode by either an added electron carrier or directly from the respiratory enzyme of the bacteria. The development of MFCs system will be also important to the advancement of alternative fuels in the future.

In addition, present day wastewater treatment plants utilize high amounts of energy and are costly to operate. According to Logan (2004), approximately $4 million is spent per year for direct cost of operation and maintaining the plant. And 25% of cost is spent for energy operation of the plant. These conventional wastewater treatment plants utilize aerobic bacteria. Organic compound in wastewater contains energy that can be harvested and converted to electricity. One of advantages is that MFCs would cut the cost of aerating activated sludge in wastewater by as much as 50% of the electricity usage, and should generate 50-90% less solids to be disposed. [16] Therefore, MFC technology has been developed as new process to offset in wastewater treatment plant to harvert electrical energy whilst treating the organic pollutants. In other word, to engineering, MFC has been expected to power the wastewater treatment plant and clean the water besides capturing energy in the form of electricity.

1.2 Thesis overview

Defining the performance of MFCs, many researchers have studied on the effect of physical or biological factors on the performance of MFCs such as: initial carbon sources (their presence in the initial medium enhanced a number of microorganism) (Kim, 2000), the rate of fuel oxidation, electron transfer to the electrode, circuit resistance, proton mass transport to the cathode through the membrane, oxygen supply and reduction in the cathode (Gil, 2003), electrode areas (size of anode and cathode electrodes affect power output) and the resistance of the electrolyte (i.e.

anode and cathode solutions, and PEM) (Zhen He, 2005). For this technology to be viable wastewater treatment method, the studies on MFC have concentrated primarily on the bacterial species able to produce electricity in the absence of a mediator, as well as the effect of open circuit voltage on power generation. Until now, there has not studied yet not only relationship between bacteria and internal resistance in MFC but also their role in electricity production besides anodophillic species which can directly transfer electrons liberated from cells to electron acceptor (anode electrode) by attaching themselves onto anode electrode surface. It seems to be suspended microorganisms play a significant role of electron carrier. However, one of important factor affecting MFC's performance is bacterial resistance which is a main reason of lossing electrical energy during electron transportation from donor to terminal acceptor. It's necessary to increase the efficiency of power output or how to decrease the factor which mainly causes energy generation loss via transportation inner MFC system. Therefore, the objectives of the study were how to improve the electrochemical activity of microbes, reduce bacterial resistance of MFCs system and enhance the electron transfer rate in anode compartment. This thesis will focus on examine some basic parameters affect MFC performance. The study has been divided into 6 experiments and using batch mode. And the aims of the experiments are listed below:

- Experiment 1: to identify that suspendid microorganism has a major role in the electricity production.
- Experiment 2: to elucidate the effect of starvation bacteria on electrical energy under two forms; starved bioflim and starved suspended microorganism.
- Experiment 3: to identify the crucial role of Fe^{3+} and Mn^{4+} in the electron transfer rate.

- Experiment 4: to observe the amount of electricity produced by different concentration of glucose and optimal of substance concentration for MFC's performance.
- Experiment 5: to determine power output as a function of concentration of substrate (eg. initial glucose concentation added)
- Experiment 6: to examine the power generated through use of different type of seed sludge (eg. activated sludge, anaerobic sludge and heated sludge).

This thesis has been organised in 4 sections. The first chapter deals with a brief overview of the content of the thesis. Chapter 2 begins by laying out some fundamental knowledgements related to MFC technology. In addition, this chapter reviews some basic theories about microbial energy metabolism, electrochemistry and kinetic modeling study and some current MFC's research. Chapter 3 described the materials for all experiments and how to set-up batch tests for each experiment. In the following chapter 4, the results of this study will be shown and discussed. Finally, chapter 5 is conclusion and further study.

CHAPTER 2
LITERATURE REVIEW

This section contains some reference principles of research and experiment to MFCs: microbial energy metabolism, electrochemistry, predictive modeling for MFC's performance (eg. specific substrate consumption rates, maximum electron production rate). In addition to, overview of current researches of MFC technology and some advantages and challenges of MFC study are also introduced in this chapter.

2.1 Microbial aspects of Microbial fuel cell

2.1.1 Microbial energy metabolism

Bacteria gain energy by the transfer of electrons and protons from a reduced substrate at a lower potential to an electron acceptor at a higher potential. The energy gained can be calculated as:

$$\Delta G = - n \times F \times E_{emf}$$

Where

n: the number of electrons exchanged

F: Faraday's constant (96485 C/mol)

E_{emf}: the thermodynamic equilibrium cell potential (also referred to as ΔV).

Bacteria can perform electrons transfer on several ways, but overall, two main classes of microbial energy metabolism are known as respiration and fermentation. Type of energy metabolism is used in the study is depended on the properties of the

bacteria and the available electron donor and acceptor. In an anode compartment of a MFC, under anaerobic condition, the degradation of organic matter provides not only CO_2 or CH_4 as the end products but also energy. Some of energy trap in form of ATP and some of lost in the form of heat. Through the ATP-ADP cycle, energy requiring for synthetic reaction of cells is provided and energy releasing from food breakdown is obtained. In the energy conversion progress, protons and electrons are liberated by redox reaction of couple $NADH/NAD^+$. Thus, the electrical energy can be achieved from which electrons pass through cell membrane. Without oxygen, if an anode is available with a higher potential than for example sulphate presents in the feed stream, the energetic gain will be much higher for bacteria that can deliver to the anode. Thus, the anode will become the preferred electron acceptor. If however the anode potential is too low, electricity production will cease and fermentation processes will start.

2.1.2 Electrons transfer in MFCs

To obtain microbial electricity production, the electrons from bacteria need extracellular electron transport towards the anode. In general, redox mediator was used, such as neutral red, methylene blue, thionine and humic acid to enhence the transport of electrons [16]. However, a number of studies have found that many different kinds of bacteria could be a capable of exogenous electron transfer without using artificial mediatiors. In these studies significantly focus on metal-reducing bacteria such as the families of Shewanella (Kim et.al. 1999, 2002), Rhodoferax (Chaudhuri and Lovley 2003), Geobacteraceae (Bond et al. 2002; Bond and Lovley 2003) or fermentative bacteria such as Clostridium butyricum (Park et al. 2001). These bacteria produced nanowires which are highly conductive or phenazine to transfer electrons [2]. Moreover, they use cytochromes on their outer membrane to transfer electron or through unknown

ways. MFCs using mixed bacterial culture also could transfer electron in the absence of mediators.

In conclusion, three major pathways can be discerned:

- Membrane associated electron transfer (Bond & Lovley 2003; Kostka et al. 2002)

- Mobile, soluble redox shuttle assisted electron transfer (Delaney et al. 1984; Roller et al. 1984)

- Nanowires or electrically conductive bacterial appendages (Reguera et al., 2005)

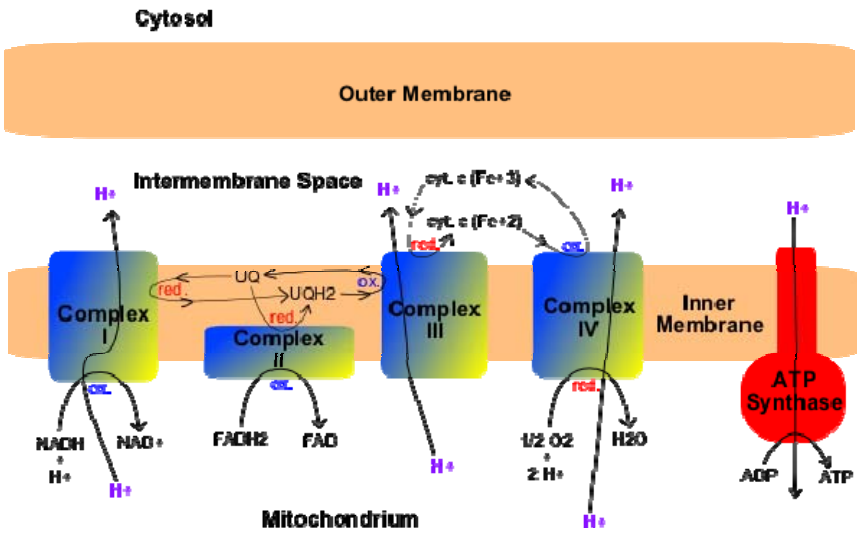


Fig. 2.1 Diagram of electron transfor chain of bacteria.

From *http://www.personal.kent.edu/~cearley/PChem/pchem.htm*

2.2 Electrochemistry

In general, the difference between the measured cell voltage and the cell emf (electron motive force) is referred to as overvoltage and is the sum of the overpotentials of the anode and the cathode, and the ohmic loss of the system. The overpotentials of the electrodes are generally current dependent and in an MFC they can roughly be categorized as: (i) activation losses; (ii) bacterial metabolic

losses; and (iii) mass transport or concentration losses. The ohmic losses (or ohmic polarization) in a MFC include both the resistance to the flow of electrons through the electrodes and interconnections, and the resistance to the flow of ions through the proton exchange membrane (if present) and the anodic and cathodic electrolytes (solution ionic strength).

Similar to that of a galvanic cell, the change in energy and entropy, the heat energy dispersed or absorbed and the useful energy produced or consumed in a MFC system is subject to the laws of thermodynamics (Rossini 1950). For this reason, limiting a thermodynamic analysis to known reversible chemical reactions that take place within the MFC simplifies calculations. However, this limits the thermodynamic analysis to that of the second law efficiency calculations instead of including first law efficiency calculations. Given a known reversible chemical reaction, a calculation of the Gibbs free energy can be expressed as (Bard, 1985, Newman 1973) [14]

$$\Delta G_r = \Delta G_r^0 + RT \ln(\Pi)$$

Where

ΔG_r = Gibbs free energy

ΔG_r^0 = Gibbs free energy under standard conditions

R = universal gas constant

T = absolute temperature

Π = reaction quotient of the products divided by the reactants

According to researchers of the Logan Group, the Gibbs free energy under standard conditions is calculated from the tabulated energies associated with the formation for organic compounds in aqueous solutions. The negative value of the Gibbs free energy is known as the maximum work of the system and can be deduced to terms of the overall cell emf as follows:

$$-\Delta G_r = W_{\max} = E_{emf} \times Q = E_{emf} \times nF$$

Where

$W_{\max}$ = maximum theoretical work

E_{emf} = potential difference between the cathode and anode

Q = charge

n = number of electrons per reaction

F = Faraday's constant

Rearranging the above equation yields,

$$E_{emf} = -\frac{\Delta G_r}{n.F}$$

And under standard conditions,

$$E^0_{emf} = -\frac{\Delta G^0_r}{nF}$$

Using the above mentioned equations, an expression for the overall electromotive force of a particular reaction at any condition can then be calculated as,

$$E_{emf} = E^0_{emf} - \frac{RT}{nF}\ln(\Pi)$$

In general, the electromotive force of the MFC, under specific conditions, can then be calculated as

$$E_{emf} = E_{cathode} - E_{anode}$$

Where

$E_{cathode}$ = electromotive force of a specific reaction taking place at the cathode

E_{anode} = electromotive force of a specific reaction taking place at the anode

The MFC second law efficiency can be evaluated by relating the theoretical electromotive force to the measured cell potential based on the assumption that the simple reactions evaluated at the anode and cathode are similar to that of the more complicated reactions involved with the bio-degradation of wastewater,

$$\eta_{MFC} = \frac{W_{actual}}{W_{\max}} = \frac{V_{measured} \times nF}{E_{emf} \times nF} = \frac{V_{measured}}{E_{emf}}$$

Where

η_{MFC} = MFC second law efficiency

W_{actual} = actual work output

$W_{\max}$ = maximum work output

$V_{measured}$ = measure voltage potential

- Polarization curve

The quantitative evaluation of a galvanic power source can be made in several ways, including cell voltage at fixed discharge current density, energy density, power density, or discharge capacity. A common way to express the performance of a fuel cell is the steady-state voltage versus current (or current density), viz. polarization curve. The open circuit voltage of an electrochemical cell can be related to the theoretical free energy change of the overall electrochemical reaction of the cell as follows:

$E_{cell} = -\Delta G/nF$

Where n is the number of electrons transferred in the overall reaction and F is the faraday constant. The variation of current can be achieved by varying the external load by different resistors. The measurement of current by an ammeter may not be accurate since the resistance of the ammeter gives additional loading to the cell. It is convenient to measure the voltage across a standard resistor with a high resistance voltmeter and calculate current by the ohm's law as $I = V / R$. A typical

polarization curve is shown on Fig.2.2. The power of the cell is simply (current x voltage) and will be at a peak value near the limiting current. Since current generated will be proportional to active surface area, it is common to plot voltage versus current density (mA/m^2) and to plot power density (mW/m^2) versus current density.

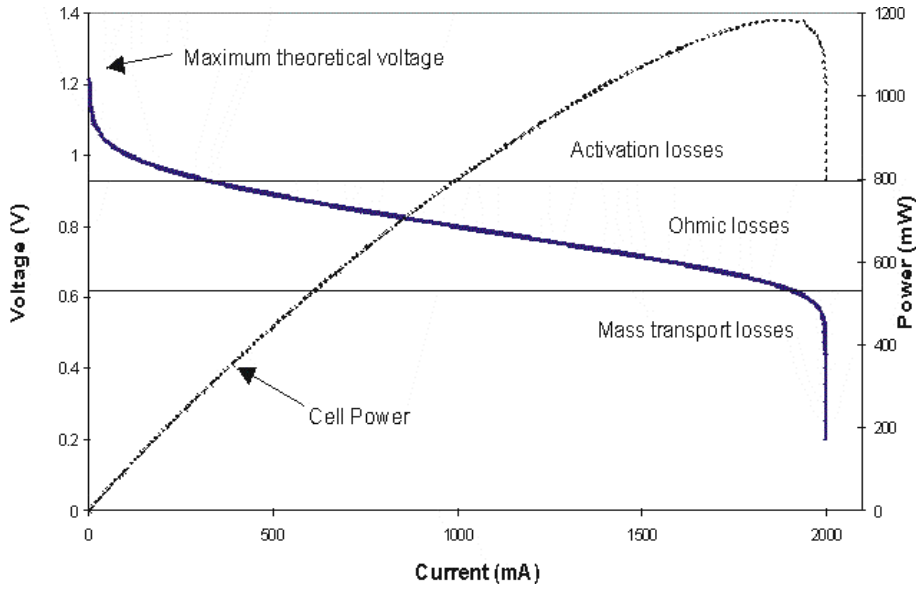


Fig.2.2 Typical polarization curve (current-potential plot*)*.

From: *http://chem.hku.hk/~fuelcell/advance.htm*

- Calculation of MFC used glucose as substrate and oxygen as reductant in cathode

A mole of any substance always has 6.022 x 10^{23} number of entities-called Avogadro's number (N). A "mole of electrons" is 6.022 x 10^{23} electrons

The charge on one electron is 1.602 x 10^{-19} coulomb (C)

$F = N.e = 96485C$

For any number of electrons per molecule pass the external circuit, n

$-n\ Ne = -n.F$ coulombs

Electrical work done = charge x voltage = $-nFV$ joules = Gibbs free energy

Thus: $\Delta G = -nFV \Rightarrow V = -\Delta G/nF$

Electromotive force (EMF) $E_{emf} = -\Delta G/nF$

If n=1

ΔG of glucose = -41.35 kJ

ΔG of oxyen = -78.72 kJ

Donor: E_{emf} = 41.35 x 1000/96485 = 0.428 V

Acceptor: E_{emf} = 78.72 x 1000/96485 = 0.815 V

ΣE_{emf} = 0.482 + 0.815 = 1.243 V (theorical) or reversible open circuit voltage (OCV) of MFC using glucose and oxygen as donor and acceptor, respectively.

The open circuit potential was estimated once a day as the maximum value that was gradually increased after the disconnection of the circuit. However, the measured MFC voltage is considerably lower due to a number of losses. In an open circuit, when no current is flowing, the maximum MFC voltage achieved thus far is 0.80 V. During current generation, voltages achieved up to now remain below 0.60 V [14].

2.3 Modeling of electrical production and cumulative hydrogen production

Modified Gompertz equation was statistically sufficient for the description of the maximum value of open circuit voltage and cumulative hydrogen production, as described by equations (2) and (3), respectively. Equation (1) expresses a typical Modified Gompertz model:

$$y = A\exp\left\{-\exp\left[\frac{\mu_m.e}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

Using experimental data for batch test, the electrical behaviour and cumulative hydrogen production were derived model curves versus time. Both the maximum

rate of electricity production and the maximum rate of hydrogen production were considered as slope of the curve during exponential growth. The curve fits were generaly based on the modified Gompertz equation. The parameters used in the below equations were estimated by fitting the observed cumulative hydrogen production from the batch test using using CurveExpert 1.3.

In MFC's study, A will be the maximum voltage ($V_{oc,\,max}$), μ_m is the maximum rate of electron pass through the circuit per hour (V_{oc} h^{-1}); λ is lag-phase time for electricity generation. The equation (1) is then rewrited as follow:

$$V_{oc} = V_{oc,\max} \exp\left\{-\exp\left[\frac{(\Delta V_{oc} / \Delta t).e}{V_{oc,\max}}(\lambda - t) + 1\right]\right\} \quad (2)$$

In case of hydrogen production, R_m is the maximum rate of hydrogen production and λ is lag-phase time for hydrogen production.

$$H_p = H_{pu} \exp\left\{-\exp\left[\frac{R_m}{H_{pu}}(\lambda - t) + 1\right]\right\} \quad (3)$$

From simulation of change in V_{oc}, maximum power output, maximum electron production rate and lag-phase time will be obtained. In the same manner, potential of hydrogen production and maximum rate of hydrogen production are also estimated. [15]

2.4 Description of MFC technology

This section provides a brief description of MFC technologies. How a MFC works and which factors is main barrier of MFC's performance.

2.4.1 What is MFC?

MFC can directly generate electricity from biodegradable organic matters. Typically, it includes anaerobic compartment and aerobic compartment. In each compartment, it has an electrode, which is called anode for accepting electrons (in

anode part) and another called cathode for donating electrons (in cathode part). An electrical wire connects these electrodes to close the current circuit. Depending on the yield of fuel cell, electrical power could be obtained via a load so called external resistance.

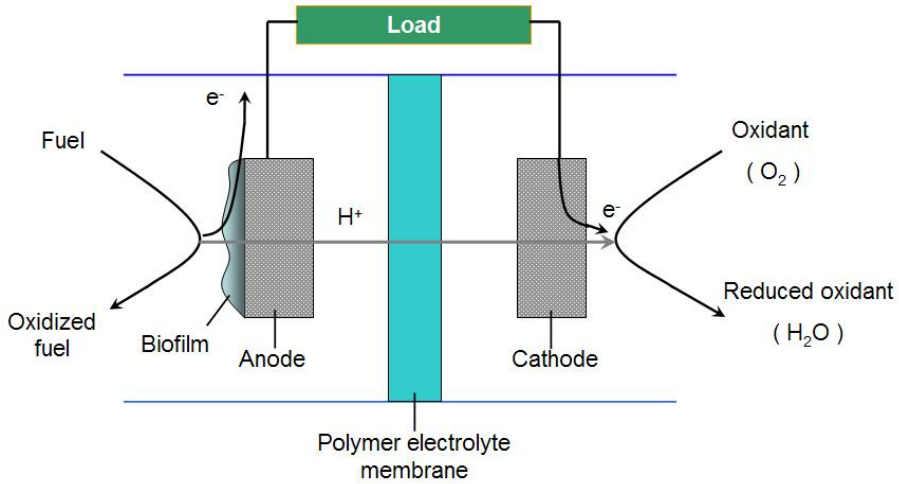


Fig.2.3 Diagram of a typical MFC

2.4.2 How does it work?

In anode chamber of MFC, bacteria grow under anaerobic condition and consume substrate to provide electrons and protons. The electrons travel to the anode and flow in ex-wire to the cathode which exposed to oxygen, current flow and potential of cell are created. At the surface of cathode (or called counter electrode), the electrons combine with protons and oxygen to form water. To achieve a high efficiency of electricity production, bacteria have to degrade organic matters under strict anaerobic condition for preventing removal of electrons from oxygen. In case of batch test, potassium hexanoferriccyanide was prefered to be used as electron acceptor associated to oxygen in cathodic solution.

2.4.3 Components of a conventional MFC

A typical MFC contains anode compartment (anaerobic condition), cathode compartment (aerobic condition), and electrical line such as load or resistor. Cathode and anode chamber are seperated by proton exchange membrane. For current flow occurrence, anode chamber requires biocatalyst (microorganism), redox mediator (electron shuttles), substrate (carbon source) and electrode (insoluble electron acceptor). Meanwhile, cathode chamber requires chemical oxidizers. Oxygen, H_2O_2, $K_3[Fe(CN)_6]$ are generally used oxidizer in catholyte.

① Anode compartment

The anode compartment is the place where stores fuel and take places metabolism reactions, trapping energy of this biological process. Under anaerobic condition, bacteria decompose substrates and liberate electrons directly or via mobile redox shuttles transferred to electrode. Therefore, an anode compartment generally includes bacteria as catalyst, fuel for bacterial metabolism, mediator for electron transfer and the electrode as terminal electron acceptor. These components of anode compartment are described in detail as the following:

- Microorganism for inoculums:

MFC operated in mixed cultures currently achieve substantially greater power densities than in pure cultures. In one recent test, however, an MFC showed high power generation using a pure culture, but the same device was not tested using acclimated mixed cultures and the cells were grown externally to the device. Community analysis of the microorganisms that exist in MFCs has so far revealed a great diversity in composition. That many new types of bacteria will be discovered that are capable of anodophilic electron transfer (electron transfer to an anode) or even interspecies electron transfer (electrons transferred between bacteria in any form).

There are two kinds of sources used for inoculum, pure culture and mix culture. With MFCs currently researched in pure culture field, various kinds of bacteria are added to the MFC system. Bacteria are classified to thousands of individual species. Those actively studied in terms of their compatibility to various mediators, coulombic yield, electron transfer rate, and regenerative abilities include: metal salt reducing bacteria *Shewanella putrefaciens* and *Psuedomonas pseudomallei* (Kim et al., 1999-2001) *Clostridium butyricum*; *Escherichia coli*; *Clostridium beijerinkii* (J. Niessen et al., 2004-2006), *Rhodoferax ferrireducens* (Chaudhuri, S.K et al., 2003), *Pseudomonas aeruginosa* (Korneel.R et al., 2005); halophilic organisms (Halme and Zhang, 1995a), and the iron-oxidizing bacteria *Thiobacillus ferrooxidans* (Bennetto et al., 1989), sulfate-reducing bacteria *Desulfovibrio desulfuricans* (Michael J Cooney et al., 1996),

In addition, mix culture such as sewage, marine sediment, wastewater from animal, industrial or domestic wastewater processing have been found that could be valueable sources of bacteria for MFCs. The communities of bacteria in mix culture also drive power production via a reaction that allows them to transport electrons from the cell surface to the anode.

- Substrate

Electrical source could be converted from carbohydrate or sugar, lipids, proteins and even yeast. These organic compounds are easy found in biomass, wastewater, and sediment etc… For long-term experiments, glucose would need to be pumped into the anode compartment as a constant source of fuel for bacterial metabolism. Over time, oxidative metabolism would gradually decrease if there is a limited availability of glucose, which would be reflected in a decreasing current output. It would be also important to ensure that an anaerobic environment exists at the anode. In order to ensure anaerobic conditions, nitrogen gas should be passed through the compartment or purging in batch mode. The presence of oxygen at the

anode would mean that the graphite electrodes would have to compete with molecular oxygen for electrons available through the electron transport chain. For a greater efficiency in electron transfer to the anode, it would be necessary to greatly reduce the concentration of dissolved oxygen. Regulation of pH at the anode is important to consider. Increased acidity of the compartment could prove detrimental to the bacterial cells. Therefore, a phosphate buffer is used, which resists changes in pH.

Below equation illustrates the principle of extracting electrons from organic substances in anode compartment of MFC system (glucose used as substrate):

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

- Mediator

As usual, the presence of artificial mediators in the medium such as methylene blue, thionine, 2-hydroxy-1,4naphthquinone or neutral red increases the efficiency of electron transfer. These dyes insert themselves across the bacterial cellular membrane, and essentially capture the electron transport chain.

- Materials of electrode

Graphite (rod, plate, and felt in shape), paper carbon, reticulated vitreous carbon (RVC) and graphite modified by noble metal (Fe, Mn, Pt, Ni, etc...) are able used as the electrode for terminal electron acceptor in anode compartment. Among of these materials, woven graphite felt is ideally used for the electrode, due to the large surface area (geometric surface area). In addition to, the graphite felt has low resistance and efficiency physical structure, which consists of an open network of interwoven fibers, providing easy access of the microbial organisms and mediator to the electrode surface.

Most results with carbon electrodes generally report power generation rates on the order of 10-100 mW/m^2. In the other hand, to improve the cathode reaction, some

researches have studied the effects of platinum-coated graphite cathode on the current yield. Jang (2003) uses ML-MFC to monitor the change in power output before and after the graphite cathode was replaced by platinum-coated graphite. Perusal of the result showed that higher current (3.2 mA) was generated when the platinum-coated graphite was used than the fresh graphite (2.0 mA), though the apparent area of the former was much smaller than the latter. To compare the effects of platinum-coating, the coated electrode was replaced with the same size fresh graphite (apparent area; 89 cm^2). The current yield of platinum-coated electrode and fresh electrode were about 20 and 13%, respectively. Similarly, Logan's group also used Pt or Pt–Ru as active cathode electrode to increase the maximum power from 19 to 33mW/m^2. Liu (2004) reported eight graphite rods were abraded by sand paper to enhance bacterial attachment and the air-porous cathode consisted of a carbon/platinum catalyst/proton exchange membrane (PEM) layer fused to a plastic support tube. Their prototype SCMFC reactor generated electrical power (maximum of 26 mW m^{-2}) while removing up to 80% of the COD of the wastewater. Some studies with different types of electrodes were listed in Table 2.1.

Table 2.1 Some studies with different type of electrode and their power output efficiency.

References	**Type of MFC**	**Culture**	**Substrate**	**Type of electrode**	**P (mW/m^2)**	**Current (mA/m^2)**
Jang et al., 2003	ML-MFC	Artificial wastewater	Glucose + Glutamate	Graphite felt / Pt-coated Graphite	1.3	6-9

Logan et al., 2004	Two chambers	Marine sediment	Cysteine	Plain carbon paper + Pt or Pt/Ru	19 to 33	
Liu et al., 2004	SCMFC	Wastewater	Acetate, lactate, and glucose	Eight graphite rod (6.15 mm in diameter and 150 mm long)	26	125
Hong Liu and Bruce E. Logan	SCMFC	Domestic wasteater	Glucose	Toray carbon paper	494-262	
G. Tayhas R. Palmore	Glass		Glucose	Pt-gauze coated – Pt black	0.015 - 0.0029	0.025
Rabaey et al., 2003	Batch Four cells	Anaerobic sludge	Glucose	plain graphite electrode (50 cm^2)	3600	
Chaudhuri et al., 2003	Two chambered vessel	Anaerobic sludge	Glucose	Graphite rod (G10)		31

Niessen et al., 2004			Starch	Woven graphite		1.3

② Cathode compartment

The basic reaction in cathode compartment using oxygen:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

A suitable candidate would be a coordination compound such as the deep red colored ferricyanide anion $[Fe(CN)_6]^{3-}$ found in the salt potassium hexacyanoferrate, $K_3[Fe(CN)_6]$. A ferricyanide cathode reaction is attractive since it is easy to assemble and has a fast electron take up. Unfortunately two problems exist with the ferricyanide cathode reaction: it does not consume the liberated hydrogen ions (which lower pH levels), and the capacity of the ferricyanide to collect electrons eventually becomes exhausted.

If potassium hexanocyanode ferric presents, two equations below illustrate the principle of reduction electrons from organic substances:

$$4Fe(CN)_6^{3-} + 4e^- \rightarrow 4Fe(CN)_6^{4-}$$

$$4Fe(CN)_6^{4-} + 4H^+ + O_2 \rightarrow 4Fe(CN)_6^{3-} + 2H_2O$$

③ Proton exchange membrane

Nafion-membrane is composed of a perfluorosulfonic acid polymer film. When in contact with water, the hydrogen proton (H^+) detaches and hops from one sulfonic molecule ($SO3^-$) to another and thus acts like an electrolyte in the presence of water. Therefore, Nafion transfers H^+ across the PEM to the cathode, but does not allow electrons to cross. H^+ yielded in the degradation of organic material by microorganisms in the wastewater (anode chamber) is then transported across the Nafion to the cathode where the protons come in contact with oxygen to form water. The sulfonic acid looses H^+, which then gives the membrane a negative charge. This negative charge attracts H^+ from the wastewater that becomes bound

on the membrane. Eventually all the negative sites will become neutralized. The Nafion-117 has a pore size of 50 Å (10-10 meters) which is popular used in MFC study.

2.5 Factors affect performance of MFCs

Relying on the diagram of electron production and transfer to electrode in anode compartment (Fig. 2.4), some major factors affecting on MFC performance can be listed as follow:

- Source of energy
- Microbial activities
- Electrochemical activity of microorganisms
- Electrochemical activity of electrode surface

These factors are discussed in part of decription of anode compartment Here just resume or extent some main content in each of factor.

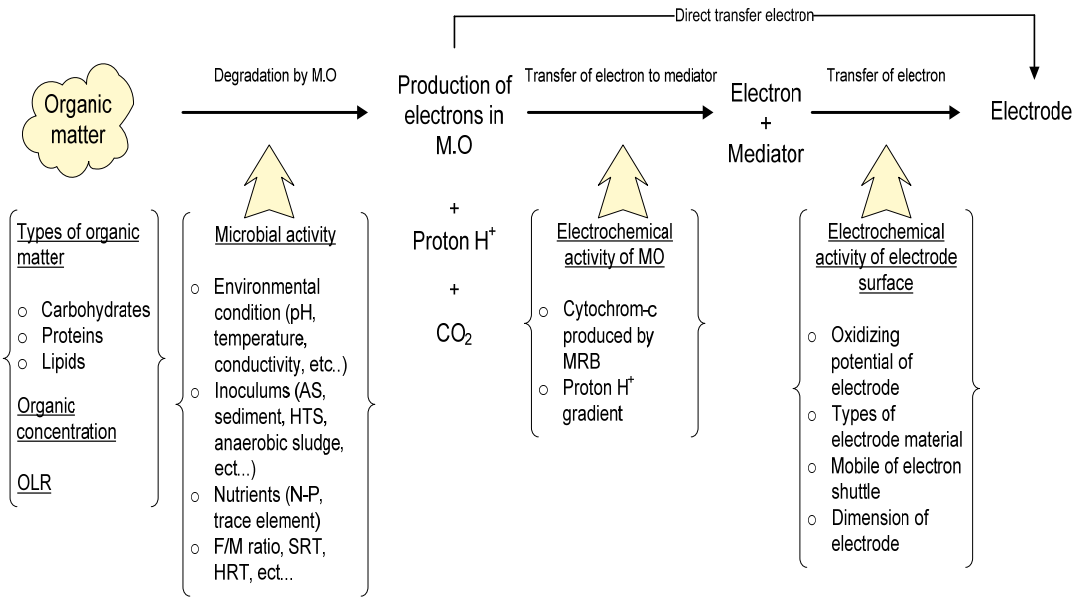


Fig. 2.4 Factors affect on electron released from bacteria to terminal negative electron acceptor (electrode)

- Source of energy

The food value of microorganism is directly related to their lipid, protein content which is major compositions of microorganisms, whereas carbonhydrate is necessary for biomass production.

Carbohydrates are a major source of energy for living organism. Carbohydrates such as starch, complex sugar, and cellulose are polysaccharides. Monosaccharide includes sucrose, glucose, fructose, mannose and galactose.These simple sugars are higher in energy content per electron equivalent than most other simple organic molecules. For this reason, microorganism frequently can obtain energy from carbohydrates by anaerobic fermentative pathways [5]. In MFC experiment, glucose is often used as energy source because of being easily degraded to smaller entities by anaerobic organism but the anaerobic pathway leads to incomplete breakdown glucose and provide a small amount of energy for the cell.

Fats and lipids (fatty acid, glycerol) have large negative free energy charge which is due to the oxidation of the highly reduced hydrocarbon radical attached to carbonxyl group of the fatty acid.

Proteins (amino acid) are necessary nutritional requirements for microorganism because they contain amino acids. [5]

Effects of various concentration of substrate such as lactate, glucose, fructose on electrical energy have been examined.

- Microbial activities and electrochemical activity of microorganisms

This factor relates to environmental condition (pH, temperature, ionic strengh…), type of inoculum (AS, AnS, HTS or sediment…), nutrient (N-P, trace elements…), F/M ratio, SRT, HRT, etc…

In this part, almost studies focus on how to decrease internal resistance of MFC and overpotentials relating potential of cell and value of open circuit voltage as below:

$E_{cell} = OCV - IR_{int}$

Where IR_{int} is sum of all internal resistance losses, internal resistance of cell depends on anodic and catholytes (ionic strength), flow of electron through the electrodes and interconection (electrode material, spacing), the resistance to the flow of ions through the PEM (if present).[14]

Open circuit voltage of MFC is assumed by:

$$V_{oc} = V_{emf} - overpotentials$$

Where overpotentials include potentials loss via: (i) bacterial metabolism losses, (ii) activation losses, (iii) concentration losses [14].

- Electrochemical activity of electrode surface

Oxidizing potential of electrode, types of electrode material or demension of electrode and mobile of electron shuttle determine the efficiency of electron transfer rate to the surface of electrode.

2.6 Research types of MFCs

In general, MFCs can be divided into different types as followed:

2.6.1 According to type of microorganism

In this type of fuel cells, MFCs can be classified into (i) Sediment MFCs, in which the potential difference between sediment and the liquid phase above is used to generate electricity, (ii) Photoheterotrophic MFCs, in which light and carbon substrate are provided to, for example, cyanobacteria which in turn can use an electrode as electron acceptor and (iii) Heterotrophic reactor MFCs, in which anaerobic bacteria use carbon substrates to generate reducing power [11]

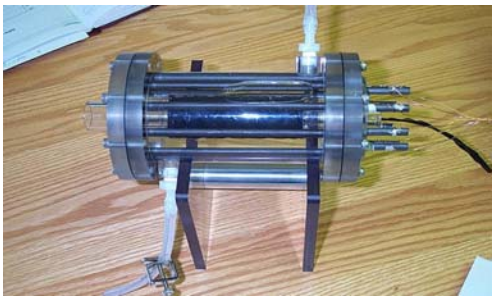
2.6.2 According to physical configuration

Several types of MFCs, which have been modified to enhance the yield of power

output, have recently gainned increased attention include: overview of several MFC designs

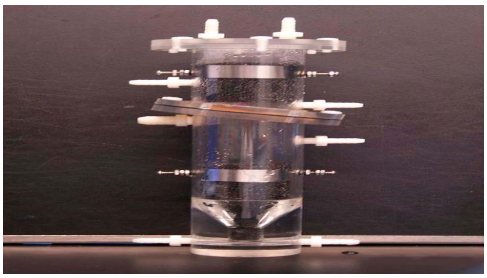
- Dual chamber MFCs (within PEM) [18] [3]
- Single chamber MFCs (remove PEM) [13] [8] [6] [12]
- Flat plate, continuous flow MFC
- Upflow reactor MFCs [7]
- Stacked configuration MFCs [1]
- Air cathode MFCs (remove PEM) [4] [11]

<This is a conventional two-chamber (so called H-shape) microbial fuel cell. In this setup, both chambers are gas sparged: one with nitrogen to maintain anaerobic conditions in the chamber where the bacteria grow (anode); the other with air to provide oxygen in solution (cathode)>

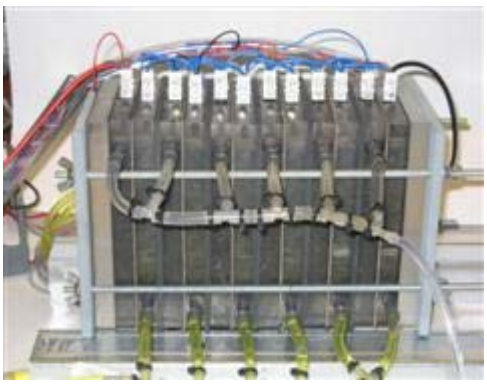


<This is the SCMFC described by Liu et al., 2004>

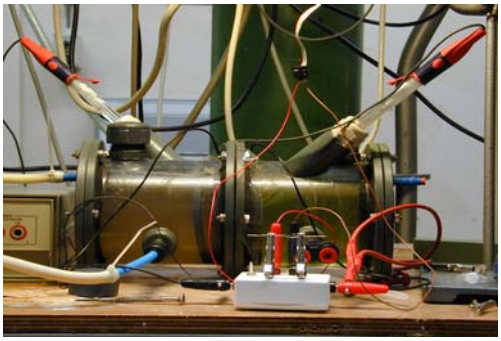
<This flat plate microbial fuel cell that operates in continuous flow mode has a proton exchange membrane sandwiched between two carbon paper electrodes. Channels are drilled to that the flow follows a serpentine path through the system. (Min & Logan, 2004)>



<Upflow tubular MFC (He et al., 2005)>



<The stacked MFCs which resulted in high power densities at enhanced voltages and currents. The series and parallel connection had no adverse influence on the activity of the microbial catalyst. This is a prommissing finding because for practical applications high voltages and currents are needed. (Aelterman et al., 2006)>



<Continuously fed MFC are used by Wageningen University (The Netherlands) >

Fig. 2.5 Overview of several MFC designs. From *http://microbialfuelcell.org/*

2.7 Applications of MFC technology

For a long time, MFCs have been interested in field of renewable energy because of following reasons: First, high conversion yield could be gained by direct exploiting electricity energy from organic matters. Second, MFCs can operate at the ambient and current bio-energy process is not sensitive on change in temperature. Third, gas-off released from MFCs need not to be treated and normally has no useful energy content. Fourth, MFCs have potential for widespread application and also to expand the diversity of fuels we use to satisfy our energy requirements [4] However, power generation obtained from these systems have just operated at laboratory scale, the efficient electricity production of MFC is still improved by optimizing its physical or electrochemical components.

- Power generation and treat wastewater

Microbial fuel cells have a number of potential uses. The first and most obvious is harvesting the electricity produced for a power source. Virtually any organic material could be used to 'feed' the fuel cell. MFCs could be installed to waste water treatment plants. The composition of organic matter in wastewater generally composed of 40-60% proteins, 25-50% carbohydrates and 10% lipids. Polysaccharides are abundant in wastewaters and include simple sugars, dissolved

macromolecules, large insoluble and heterogeneous exopolymers. These sources of fuel for bacterial metabolism are a large potential for electricity generation. Especially, in the wastewater treatment plant (WWTP), its application in WWTP has an economic potential when electricity could be generated while organic pollutants are treated. Thus, MFC is considered the new type of renewable energy technology in which electricity can be generated by bacteria as biocatalyst. This system can generate electricity from many kinds of organic material available in some sources such as municipal wastewater, sewage, marine sediment, animal-waste processing, industrial wastewater even corn stove biomass.

- Biosensor

Since the current generated from a microbial fuel cell (MFC) is directly proportional to the strength of wastewater used as the fuel, an MFC can be used to measure the strength of wastewater [9]. The strength of wastewater is commonly evaluated as biochemical oxygen demand (BOD) values. BOD values are determined incubating samples for 5 days with proper source of microbes; usually activate sludge collected from sewage works. When BOD values are used as a real time control parameter, 5 days' incubation is too long. An MFC-type BOD sensor can be used to measure real time BOD values. Oxygen and nitrate are preferred electron acceptors over the electrode reducing current generation from an MFC. An MFC-type BOD sensors underestimate BOD values in the presence of these electron acceptors. This can be avoided inhibiting aerobic and nitrate respirations in the MFC using terminal oxydase inhibitors such as cyanide and azide [17]. This type of BOD sensor is commercially available.

- Food powered Robot Applications

In the field of biomechanics: “Gastrobot” is a bioelectromechanical machine which uses MFC technology to supply power source to mobile robot operation [19]. The term Gastrobots –“a robot with a stomach” was coined in 1998 by the Institute’s

director; Dr. Stuart Wilkinson in which system has the operational power source comes from the digestion of real food through bacterial metabolism.

There are already some models of Gastrobots

- Slugbot (Robot devourer of slug)
- Chew Chew of Wilkinson and his team (Fig.2.6)
- Ecocobott I, II (Fig.2.7)

Fig.2.6 "Gastrobot Chew-Chew" - the robot composes a "stomach", "lung", gastric pump, "heart" pump, and a six cell MFC stack, Ti plates, carbon electrodes, PEM and *E.coli* as biocatalyst.

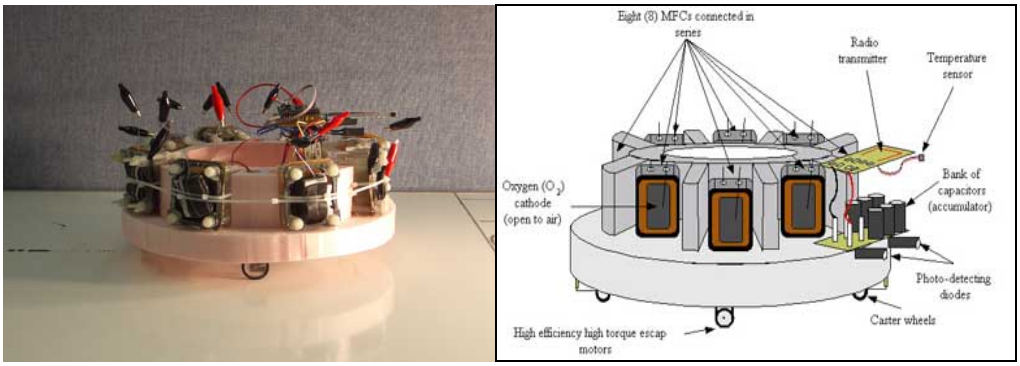


Fig.2.7 EcoBot II was installed 8 MFCs to produce electricity by digesting the exosquelettes (or wraps lasts external of an arthropod) of the flies containing of sugar. From: *http://www.ias.uwe.ac.uk/Energy-Autonomy-New*

CHAPTER 3
MATERIALS AND METHODS

3.1 MFC design

Main experimental apparatus for assembly MFC batch are:

- ✧ Plastic bottles (in 1000 mL capicity)
- ✧ PEM: Nafion 117, 10.05 cm^2, (thickness, 7mil=177.8μm)
- ✧ Electrode: graphite felt, GF National® (dimension,1x12x5,cm)
- ✧ Copper wire, 5kΩ±5% variable resistor were used as external resistance

For the study, dual-chamber MFCs type was made for experiment factors following way: Two plastic bottles, attaching a polyvinyl chloride tube (inner diameter 3.3cm, length 5cm) on the wall of each bottle, were prepared. A rubber ring on the end of each PVC tube was placed and a hydrogen selective permeable membrane (Nafion 117, Dufont) was fitted between the rubber rings, and then the tubes were clamped. For the electrodes, a graphite felt sheet (superficial area 154cm^2) was put into each bottle. The electrodes were connected with copper wires containing a variable resistance (maximum 5kΩ). One of the bottles containing the electrode was used for anode chamber and the other was for cathode chamber. Fig.3.1 shows the photograph of MFC used in this study.

Fig. 3.1 MFC batch in this study

3.2 Seed sludge and culture medium

An activated sludge (AS) was taken from S municipal wastewater treatment plant used as seed sludge. The nutrient medium for the anode chamber and the catholyte solution for the cathode chamber contained were prepared by adding chemicals listed in Table 3.2. In order to prevent abrupt pH change in the anode chamber, the phosphate buffer solution including K_2HPO_4 and KH_2PO_4 was also added into 1 litter of deionized water. Glucose was used as the substrate in the anode chamber. Table 3.1 shows in the detail the function of each component used in all batch experiments. In cathode compartment, artificial electron mediator, 100mM hexacyanoferrate solution was used as effective catholyte oxidant instead of perohydroxide for further increases in power density. Nevertheless, it must be replaced after ferricyanide to be converted ferrocyanide. All solutions were prepared with deionized water, kept at room temperature 22~25^{o}C and mixed of anode chamber was around 90~120 rpm.

Table 3.1 Inoculation and stock solution were prepared in batch test

Components	**Function**
NH_4Cl, $CaCl_2$, KCl NaCl, $MgCl_2$, Yeast extract	Nutrient for bacteria growth
Glucose	Substrate
K_2HPO_4, KH_2PO_4	Phosphate buffer solution
Activated sludge	Microorganism source
$K_3[Fe(CN)_6]$	Electron acceptor in cathode (positive terminal)

Table 3.2 Component chemicals were prepared to add into anode and cathode chamber

Anode solution, (g/L)		**Cathode solution, (g/L)**	
KH_2PO_4	2.50	NaCl	5.84
$MgCl_2$ x 6 H_2O	1.65	K_2HPO_4	1.26
NaCl	2.00	KH_2PO_4	0.42
NH_4Cl	2.00	$K_3[Fe(CN)_6]$	32.92
$CaCl_2$ x 2 H_2O	0.25		
Yeast	0.13		

3.3 Basic characteristic experiments

Before feeding, some characteristics of seed sludge such as SVI (sludge volume index), MLSS (mix liquor suspended solid), VSS (vapor suspended solid), COD,

and pH were tested. After feeding, 100 mL sample was obtained from each MFC to test pH, $SCOD_{Cr}$ (suspended chemical oxygen demand), VSS (volatide suspended solid), conductivity and salinity. All batch tests were carried out in a temperature-controlled room at 25°C. The completely mixed state in the batch MFCs was maintained using a stirring machine at 90 ~ 120 rpm.

The MLSS was determined at 105°C and VSS at 550^{o}C according to the standard method (APHA 1995). Analysis of SCOD in the culture filtrate (filtration through a 0.2-μm-pore-size membrane) was performed using the $K_2Cr_2O_7$ digestion method (APHA 1995).

Gas Chromatograph (GC, Gow-Mac, Instrument Company) test was made in study about effect of type of inoculum on electricity generation. 5mL sample was got from gas collector of each batch for test of composition of biogas.

3.4 Experimental procedure and initial condition

The study was divided into 6 parts to test one by one factor as listed in Table 3.3. Initial condition was made the same as described in item 3.2 above for every experiment.

Table 3.3 Factors were examined in each experiment.

Experiments	Aims
1	Effect of suspended microorganisms on the performance of MFCs
2	Role of biofilm and VSS affect electricity production ① role of biofilm on electric output in MFC

	② role of suspend MO on electric output in MFC
3	Effect of starved condition on electricity generation
4	Effect of soluble metal ions (eg. Fe^{3+}, Mn^{4+}) in the anode compartment on the electron transfer of microbial fuel cell
5	Effect of glucose concentration on the electricity generation in MFC
6	Effect of different inoculum (e.g. activate sludge, anaerobic sludge and heat-treated anaerobic sludge) on power output

3.4.1 Experiment 1

To test the effect of VSS concentration on electricity generation, five MFCs were set-up to make the same environmental condition for microorganism activity.

Table 3.4 The quantity of component added to anode compartment in experiment 1

Component	**MFC 1**	**MFC 2**	**MFC 3**	**MFC 4**
Glucose	0.468 g	0.468 g	0.468 g	0.468 g
Seed sludge (AS)	500 ml	250 ml	125 ml	62.5 ml
DW + medium	500 ml	750 ml	875 ml	937.5 ml

Table 3.5 Initial condition after feeding of MFCs in the experiment 1

Reactor	pH		Conductivity(mS)		VSS(g/L)	$SCOD_{Cr}$ (mg/L)
	Anode	Cathode	Anode	Cathode		
MFC1	7.17	7.08	5.70	27.80	2.52	296.1
MFC2	7.19	7.09	5.43	28.00	1.28	273.9
MFC3	7.20	7.09	5.32	28.00	0.64	288.7
MFC4	7.19	7.07	5.24	28.20	0.32	271.4
Blank	7.20	7.05	5.26	28.50	0.40	244.3

3.4.2 Experiment 2

For testing the effect of biofilm conformation on the electricity generation and its role of VSS and biofilm in electron transfer, this experiment was divided into 2 steps. First is to biofilm form on the surface of graphite in abundant nutrient, the second is to add AS after medium is replaced. The quantity of each agent was similar to experiment 1.

Table 3.6 The quantity of component added to anode compartment in experiment 2

Component	MFC 1	MFC 2
Glucose	0.468 g	0.468 g
Seed sludge (AS)	500 ml	500 ml
DW + medium	500 ml	500 ml

Table 3.7 Initial condition of MFCs in the experiment 2

Content	pH		Conductivity(mS)		VSS (g/L)	$SCOD_{Cr}$ (mg/L)
	Anode	Cathode	Anode	Cathode		
Before biofilm formation	7.24	7.12	4.57	26.10	4,07	583.0
Biofilm only	7.13	7.07	6.06	33.40	0.0	200.3
VSS+Biofilm	7.12	7.06	6.04	33.8	3,01	354.7

3.4.3 Experiment 3

To test the effect of starved condition on ability of microorganism activity, the experiment was divided into two steps compirsed total 4 batch-MFCs, three MFCs for test different period of starvation, another has not substrate in all time. At the first step, 4 batch-MFCs were set-up and monitored until they had the similar power output. It means all MFCs had already reached same performance of microorganism activity. The second step started when the voltage of all MFCs reached stable stage, the medium in anodes were replaced and AS was added again and after 2, 4, 8 days, MFC-1, MFC-2, MFC-3 were supplied glucose, respectively. However, MFC-4 (infinitive starvation) was not supplied glucose after replacing medium.

Table 3.8 The quantity of component added to anode compartment in experiment 3

Component	**MFC 1**	**MFC 2**	**MFC 3**	**MFC 4**
Glucose	0.468 g	0.468 g	0.468 g	0.468 g
Seed sludge	500 ml	250 ml	125 ml	62.5 ml
DW + medium	500 ml	750 ml	875 ml	937.5 ml

Table 3.9 Initial condition of MFCs in the experiment 3

Starvation time (day)		**pH**		**Conductivity (mS)**		**VSS (g/L)**	**SCOD (mg/L)**
		Anode	**Cathode**	**Anode**	**Cathode**		
Start-up	2	7.22	7.13	4.51	26.60	4.07	-
	4	7.22	7.14	4.40	26.70	4.07	-
	8	7.23	7.15	4.46	26.60	4.07	-
	~	7.21	7.13	4.42	26.80	4.07	-
After biofilm formation	2	7.11	7.08	6.21	33.60		266.8
	4	7.12	7.07	6.21	33.60		245.8
	8	7.11	7.07	6.18	33.80		242.3
	~	7.11	7.08	6.19	33.90		217.8

3.4.4 Experiment 4

To investigate roles of ferric and manganese ions on the electron transfer to the anode, metal compounds such as $MnSO_4.H_2O$ and $FeCl_3.6H_2O$ were added to the

anode medium before flushing by nitrogen gas. However, in the control MFC, any ferric or manganic compounds were not added. The quantity of other components was filled as Table 3.10.

Table 3.10 The components of anode compartment in experiment 3

Components\MFC	**Fe**	**Mn**	**Control**
Glucose (g)	0.234	0.234	0.234
PBS (mL)	50	50	50
Medium (mL)	50	50	50
Inoculum (mL)	500	500	500
DW (mL)	400	400	400

Table 3.11 Initial condition of MFCs in the experiment 4

MFC	**Anode compartment**						**Cathode compartment**		
	VSS (g/L)	**SCOD (mg/L)**	**$FeCl_3$ (mM)**	**$MnSO_4$ (mM)**	**pH**	**Cond. (mS)**	**$K_3[Fe(CN)_6]$ (mM)**	**pH**	**Cond. (mS)**
Fe^{+3}	2,360	335.6	1	-	7.07	8.52	100	7.04	32.6
Mn^{+2}	2,040	320.2	-	5	7.06	7.83	100	7.06	32.4
Control	2,240	335.6	-	-	7.06	6.8	100	7.06	32.7

3.4.5 Experiment 5

To determine power generation as a function of substrate concentration, 6 MFCs were also inoculumed as presented in Table 3.12. The medium in both anode and cathode was replaced each time glucose was added to adjust pH in anode and prevent the exhaustion of ferric reduction in cathode. Table 3.13 provides the initial

condition of six MFCs in the experiment 5.

Table 3.12 Component of agents were added to anode compartment in experiment 5

Component \ MFC	**MFC 1**	**MFC 2**	**MFC 3**	**MFC 4**	**MFC 5**	**MFC 6**
Glucose (g)	0.08	0.13	0.21	0.42	0.84	1.68
BS* (mL)	50	50	50	50	50	50
Medium (mL)	50	50	50	50	50	50
Inoculumn (mL)	500	500	500	500	500	500
DW (mL)	280	280	280	280	280	280

**Buffer solution: $NaHCO_3$ -500 mg $CaCO_3$/ L instead of phosphate buffer used in previous tests*

Table 3.13 Initial condition of MFCs in the experiment 5

Content	**Anode compartment**				**Cathode compartment**	
	VSS (g/L)	SCOD (mg/L)	pH	Cond (mS)	$K_3[Fe(CN)_6]$ (mM)	pH
MFC1	2.78	82.15	7.71	6.02	100	7.13
MFC2	2.86	70.42	7.71	6.06	100	7.14
MFC3	2.70	74.33	7.71	6.07	100	7.14

MFC4	2.78	78.24	7.71	6.06	100	7.17
MFC5	2.84	93.89	7.72	6.05	100	7.14
MFC6	2.84	93.89	7.76	6.07	100	7.13

3.4.6 Experiment 6

To test electrical energy generated from different microorganism sources under the same environmental conditions, three kinds of seed sludge were used to inoculation in this topic. This experiment was set with 3 batch-MFCs. Heat treated sludge was treated by mean of boiling anaerobic sludge 2 hours at 100^{o}C (according to Logan, 2005). Table 3.14 below shows chacracteristics of activated sludge and anaerobic sludge before seeding. Initial condition of six MFCs after feeding inoculum and medium was presented in Table 3.16

Table 3.14 Basic charateristics of inoculums

Type of inoculum	**pH**	**Cond. (mS)**	**$SCOD_{Cr}$ (mg/L)**	**$TCOD_{Cr}$ (mg/L)**	**TS (g/L)**	**VS (g/L)**	**VSS (g/L)**
Activated sludge	6.92	4.11	170.21	6524	7.60	5.62	5.30
Anaerobic sludge	7.28	7.12	775.41	-	40.53	29.87	22.13

Table 3.15 Component of agents were added to anode compartment in experiment 6

Condition \ MFC	1-Activated sludge	2-Anaerobic sludge	3-Heated sludge
Glucose (g)	0.42	0.42	0.42
Alkalinity buffer (mL)	50	50	50
Medium (mL)	50	50	50
Inoculumn (mL)	500	130	130
DW (mL)	280	750	750

Table 3.16 Initial condition of MFCs in the experiment 6

Content/ MFC	Anode				Cathode		
	VSS (g/L)	SCOD (mg/L)	pH	Cond. (mS)	$K_3[Fe(CN)_6]$ (mM)	pH	Cond. (mS)
1-AS	2.64	458.02	7.52	5.64	100	7.18	32.8
2-AnS	2.48	324.98	7.53	6.2	100	7.18	33.0
3-HT	2.24	884.17	7.52	6.11	100	7.18	32.9

3.5 Monitoring and data analysis

3.5.1 Operation

Experimental apparatus for operating MFC

- Digital Multimeters/Data Acquisition-Model 2700 (Keithley Instruments, Inc., Cleveland, USA) for acquisition data of electric potential or current (total 24 channels)
- Magnetic stirrer (RH Digital KT/C, IKA®) and stirring bar

- Ag/AgCl reference electrode, Orion 90-92, Thermo Electron corporation
- Platinum Wire counter electrode, CHI115, CH Instruments, Inc.
- Platinum Working Electrode, CHI102, CH Instruments, Inc.

The digital multimeter (DMM, Keithley, 2700) was connected in parallel with the resistance (set at 250Ω for all tests). A computer was connected to the RS-232 port of DMM for the monitoring of electric outputs from the MFC.

- Data was recorded on DMM every 3 minutes and transcripted using *Keithley XLINX Startup Software*
- Recording value of Voltage (using channel 112,113, 114…120) and Current (using channel 121, 122)
- V_{oc} (open circuit potential- the voltage arcoss the terminal of a MFC that doesn't have anything connected to it) was collected by connecting the DMM directly to the two electrode lines of MFC. The measurement should be taken when voltage maintained at the stable state.
- I_{sc} (short circuit current-measure the amount of electron negative pass through the ampere metter that its impedance is very small) was measured by connect two leads of DMM (channel 121or 122) to the two terminals of MFC. The measurement should take in a short-time to avoid exhausting of electrons produced.
- Polarization curve: to determine the maximum power output as a function of resistance. A polarization curve was obtained by changing external resistance from 0 to 5kΩ, measuring voltage and current at many points in order to calculate current density and power density. From that data a power density curve was generated for each MFC. The power density curve allows us to do scale-up design calculations by calculating what electrode area would be supply an optimal power.

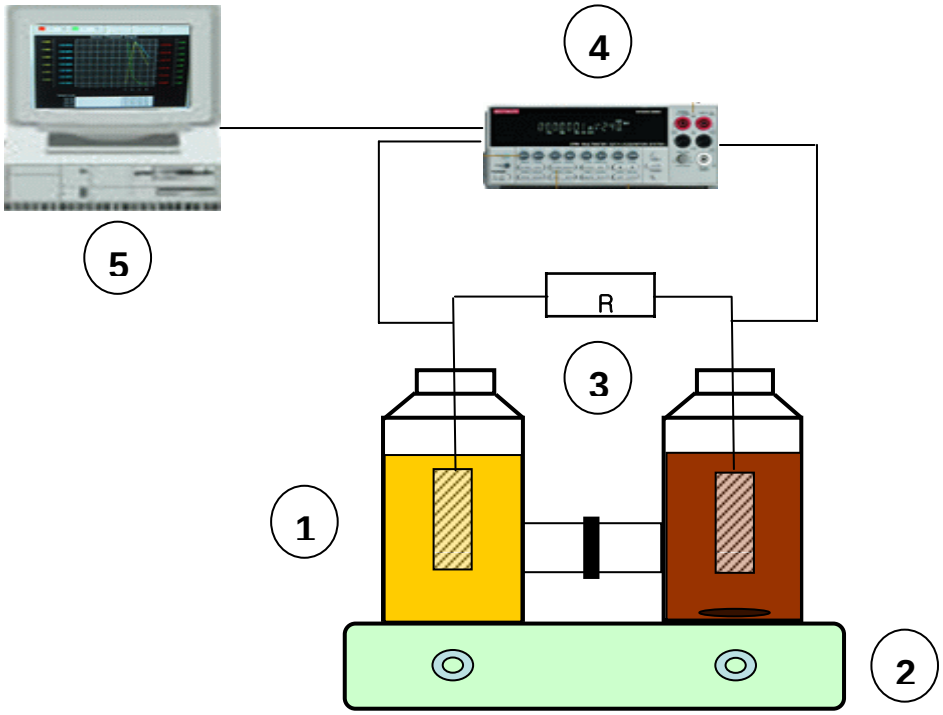


Fig.3.2 Schematic of MFC system in this study. (1) Dual chambered batch (or called H-shape) (2) Hot plate/stirrer (3) Variable resistor, 5kΩ±5% (4) Digital Multimeter/Data Acquisition, Model 2700, Keithley Instruments Inc. (5) Computer

3.5.2 The effeciency of MFC calculation

The power transferred to the external resistance from the MFC and the energy was calculated with following equation:

$$P\ (Watt) = I \times V$$

Where: I is the current (A), V the voltage (V), R the external resistor (Ω), Q the charge (C) and t the time (second).

The power density was obtained from the division of the power by the superficial area of the anode (154cm^2):

$$P_d\ (W/m^2) = P/\alpha$$

Where: α is geological surface area of electrode.

The internal resistance of the MFC system was estimated by the equation:

$$R_{int}\ (\Omega) = (V_{oc} - V)\ x\ R\ x\ V^{-1} \text{ or } R_{int}\ (\Omega) = V_{oc}\ x I^{-1}_{sc}$$

Where: V_{oc} is open circuit potential and I_{sc} is the short circuit current. The open circuit potential was estimated once a day as the maximum value that was gradually increased after the disconnection of the circuit.

The charges were calculated by integrating current over time:

$$Q = \int I\ x\ dt$$

The Coulomb efficience of MFC was calculated as follow equation:

$$CE = Q/Q_{Ti}$$

Where:

Q_{Ti} is the theoretical amount (=4.F.S.v/32) of coulomb from the substrate, where *F* is the Faraday (96,845C/mol electron), and S_i is the concentration of COD removal (g/L), v is the volume (L) of the medium in the anode chamber. In this study, glucose was used as the substrate, so the values were followings: Q_{Ti} = 96485 (C/mol.e) x SCOD (g/L) x 4 (mol e/mol O_2) x 0.9 (L) / 32 (g O_2/mol O_2).

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Effect of VSS concentration on electricity generation

4.1.1 The maximum power output

The results obtained from the calculation of power density are shown in Fig. 4.1. This experimnent lasted 240 hours. Power density was calculated by dividing power output to geometric area of graphite electrode (approximately 0.154 m^2). In the figure there is a clear trend of increasing power density with 2.52 gVSS/L of suspended microorganism in anode compartment. In case of smaller VSS concentration (1.28, 0.64, 0.32 gVSS/L), the power density are similar. Unlike expected result, it was hypothesized that electricity generation would changed in proportion to VSS concentration. However, there was not significant different between them. In other words, the power output density not changed according to level of VSS concentration. It only showed clearly the higher power density of MFC-1 which was supplied 2.52g VSS/L. This finding was unxpectected and susgests that the optimal value of VSS concentration of MFC in this study. Based on this result, VSS concentration of anode solution was adjusted to 2.50 g/L in all other experiments.

To determine the maximum value of power output as the function of external resistance, a range of 0-5000Ω was employed when voltage reaches a steady state comparatively. MFC was fed 2.52 g VSS/L showed a highest value of power density and maximum power density (76.89mW/m^2) was obtained at the resistance of about 90Ω from the polarization curve.

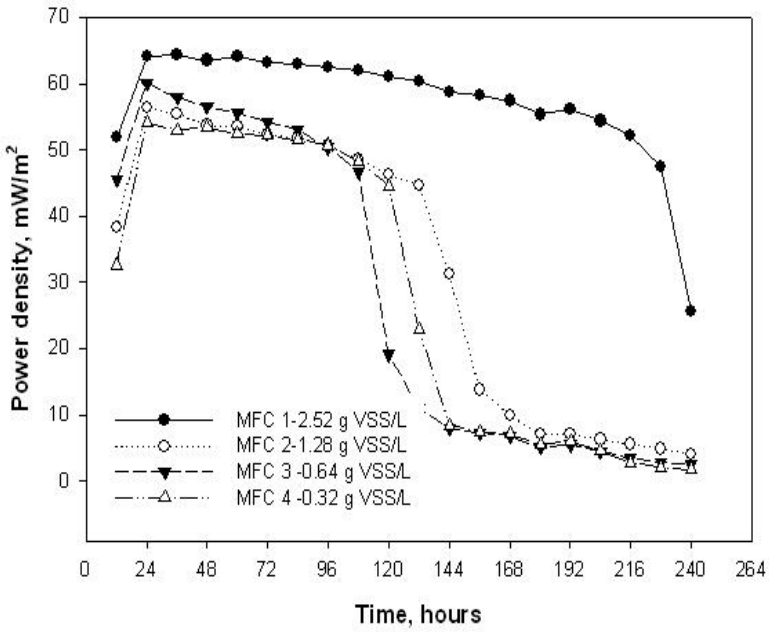


Fig. 4.1 Effect of difference of VSS concentration on power density of MFC

Effect of discharge current on cell voltage and power was showed by the polarization curve. The curve showed that ohmic loss dominated at all current densities (linear voltage decay and parabolic power density). As shown in Fig.4.2, the maximum power density reach at the 0.35V of voltage and 250 mA/m^2 of current density corresponding to 90 Ω of external resistance (Fig.4.2). This means, MFC could be obtained maximum power density by decreased value of external resistance to around 90Ω. In addition, the power density curve allows us to do scale-up design calculations by calculating what electrode area would be needed to power a MFC system.

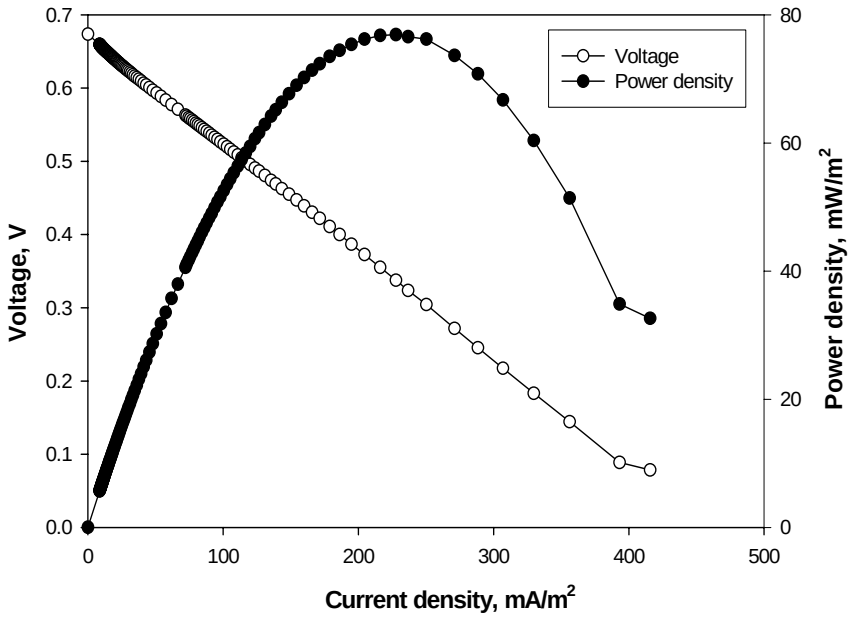


Fig.4.2 Polarization curve of MFC added 2.52g VSS/L in anode compartment.

4.1.2 Internal resistance

There are some reasons for increasing potential loss during performmance of MFC. Ohmic loss is one of those causing lost in potential. In ohmic loss, it has been reported that internal resistance of the system is considered to be the important factor to limit the performance of MFC. It was primarily a function of the proton exchange system which relative to the membrane resistance, and depended on both the resistance of the electrolyte between the electrodes (Min, 2005). In order to reduce the internal resistance, several MFC studies have reported in different methods, for example, Liu (2004) omitted the membrane and used pressed carbon paper as the separator, but this method allowed poisoning of the cathode catalyst, reduced electrode spacing (Liu, 2005); Min (2005) used salt-bridge MFC. As shown in Fig.4.3, the smaller value of VSS concentrations, the higher R_{int} was recorded. An increase in the internal resistance of the MFC might be due to i) Diffusion of hydrogen ions in the anode compartment, reducing the rate of transfer

across the PEM, ii) Formation of products which could coat the electrode or the proton exchange membrane, iii) Cells dying over time as a result of changes in pH, iv) Reduced availability of electrons for transfer to the electrodes as a result of decreasing concentrations of glucose, v) Denaturation of the electron mediator, vi) Increasing transfer of electrons to molecular oxygen (Gusphyl A. Justin, 2001). In general, there are two ways to decrease the internal resistance: one is to raise the solution conductivity by increasing the ionic strength (IS) (Liu, 2005), and the other is to decrease electrode distance and area (Ghangrekar, 2006). From this finding, we can decrease internal resistance of the MFC system based on VSS concentration. In conclusion, the resuslt of this topic indicated that higher VSS concentration, the lower internal resistance and higher power output was achieved.

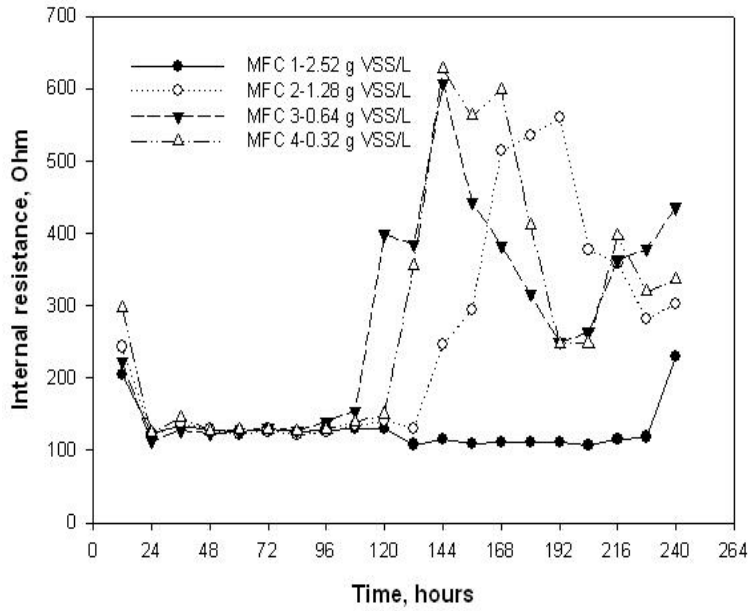


Fig.4.3 Mean value of internal resistance of MFCs according to VSS concentration

Table 4.1 Power density, V_{oc}, internal resistance and coulomb efficiency for MFCs with different VSS concentration.

Experiment 1	MFC	P_d[a], mW/㎡	V_{oc}[b], V	R_{in}[a], Ω	% CE
VSS concentration	1-2.52 g VSS/L	53.68	0.77	147.04	59.44
	2-1.28 g VSS/L	29.39	0.70	257.91	40.37
	3-0.64 g VSS/L	25.39	0.71	290.48	35.79
	4-0.32 g VSS/L	25.17	0.69	278.93	35.92

[a] *mean value*

[b] *maximum value*

4.2 Effect of attached MO and suspended MO on electricity generation

4.2.1 MFC performance before and after biofilm formation

To determine the role of attached MO (called biofilm) on the electricity generation, this experiment was divided into two phases. The first phase was to make the biofilm on the surface of electrode and the second to perform the effect of attached MO and suspended MO. The second phase started after the voltage output maintained at steady stage. Anode solution was then refreshed to supply new nutrient and fuel. Fig.4.4 illustrated MFC performance before and after biofilm formation. Initial value of voltage in second phase was higher than of first phase. It is explained that biofilm was available on the surface of the electrode, so it took a shorten acclimation time to reach maximum value of voltage than in first phase. However, the maximum voltage in second phase could not reach to the same voltage like before. Its reason may be the absence of suspended MO in anode medium. Therefore, it's necessary to conform the effect of suspended MO accompanies with biofilm on electricity generation. This result indicated that biofilm is one of factors affecting on the electron transfer rate. In some papers, they

reported that biofilm has a role of multilayer which create a shorten electron-transfer route so that electrode accepts electron transferring easily.

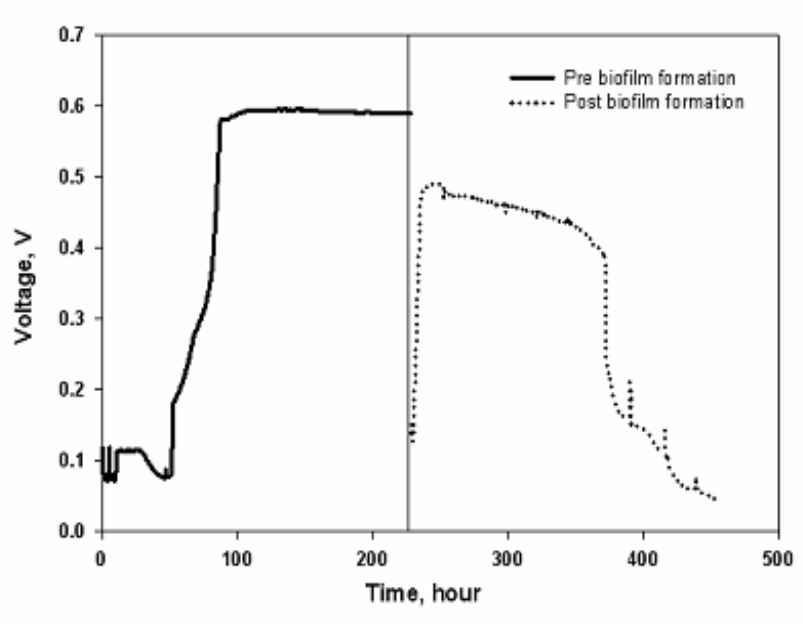


Fig. 4.4 Biofilm causes different initial and stable value of electric output in MFC.

4.2.2 Role of biofilm and suspend MO in generation of electricity

In order to determine the role of suspended MO in generation of electricity, one more test was performed by adding again inoculum after replacing anode medium. Fig.4.5 shows the electric output in two cases. In case of the suspended MO effect, MFC performance was improved slightly for 150 hours and maintained for long term at stable state. It means that suspended MO releases the electrons through degradation of substrate and combines with biofilm to increase yield of electric generation. In other words, both of them all produce electric energy while composed organic matter.

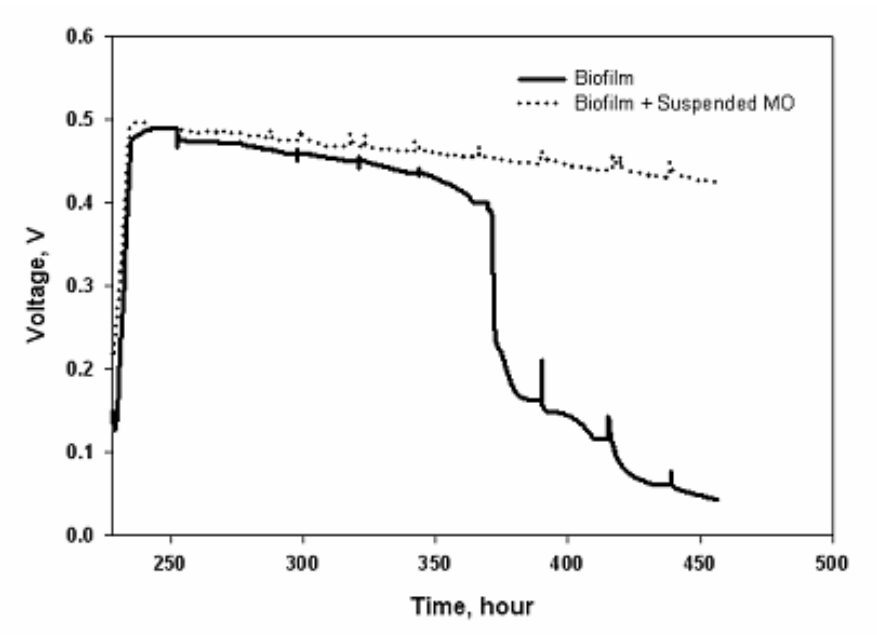


Fig.4.5 Biofilm formation available on the surface of GF, the performance of MFC was enhanced in the presence of suspended MO (seed sludge was added again after refresh medium in anode)

4.3 Effect of starved condition on microbial activity of biofilm and suspend microorganism

Changes in the voltage under varying starvation time are shown in Fig.4.6. With 2 day starvation, the result showed that voltage rapidly increase to high value but maintain just 40 hours. In case of 4 day starvation, the same performance displayed after adding glucose and also lasted high value of power output in short time. The result showed that starved biofilm affected power on external resistor. Further more, the longer starvation time the shorter steady stage. It means starvation time caused by attached microorganism was active to survive when substance for microoganism growth exhausted. With 8 day starvation, the period of time was too long for MO survice. Thus, almost attached microorganisms are died. Starvation affected biofilm detachment causing low electricirty generation after nutrient depleted completely.

Starvation effect on power output by biofilm didn't get higher yield than no starvation. In the control experiment, without any substrate in the MFC, attached MO was able to produce a stable voltage of 0.3~0.4V during 50 hours (data not shown).

On the other hand, MFC starved for 8 days did not change clearly in voltage output. The weak peak of voltage increased when adding glucose caused by amount of MO alomost degraded in culture. It may be too long time for MO to survive with deficient fuel condition. In additon, the longer starvation time the shorter steady stage time was. From the result, 4 day starvation may be an optimal condition. This study indicates that starvation has a negligible effect on electricity generation of MFC.

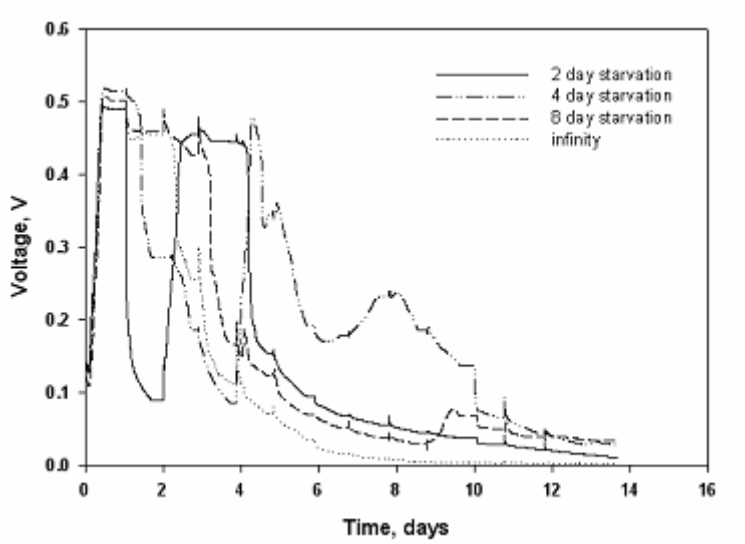


Fig.4.6 The changes in electric output during operation under various starvations

In conclusion, the observed effect of different starvation time on electricity generation in this study was not significant. Starvation could not become a factor of remarkable improvement in power output. Table 4.2 presents the small mean value of power density, maximum V_{oc}, and coulomb efficiency obtained from this experiment.

Table 4.2 Power density, V_{oc}, internal resistance and coulomb efficiency for MFCs with different starvation time.

Experiment 1	MFC	P_d[a], mW/㎡	V_{oc}[b], V	R_{in}[a], Ω	% CE
Starvation condition	2 days	9.85	0.32	558.73	14.42
	4 days	12.67	0.50	535.90	20.20
	8 days	10.40	0.38	720.54	15.52
	Inifition	7.56	0.27	1,829.98	-

4.4 Effect of Fe^{3+} and Mn^{4+} ions in the anode compartment on the electron transfer

4.4.1 Power output in the presence of Fe^{3+} and Mn^{4+} ions

The voltages of three MFCs were shown in Fig.4.7. After reaching to maximum value of 0.34 V, the voltage of control MFC gradually dropped to less than 0.2 V. In most case, changes in voltage of substrate reduced with time because of microbial exhaustion or substrate depletion. Other reason for this could be the performance of biofilm bacteria at anode electrode. When bacteria flow through anode compartment, a part of bacteria leaves and attaches to the surface of graphite to form a biofilm. The competition of biofilm bacteria with anode oxidation reaction leads to reduce power of the fuel. However, in this work, with the help of Fe and Mn ion, the voltage could be maintained at higher steady value and two MFCs showed much higher potential than control MFC over 5-week operation. In addition, Mn-MFC had three times to raise the electrical output and maintained at around 0.48 V for 10 days whereas Fe-MFC could achive to maximum of 0.52 V.

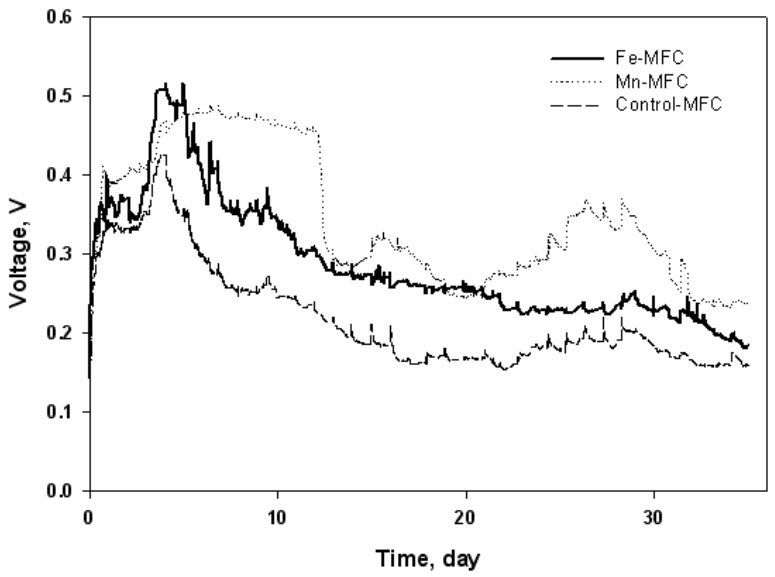


Fig.4.7 Electricity generation of MFCs over time under a load of 250Ω (main figure); solid line: Fe-MFC, dotted line: Mn-MFC, medium dash line: control MFC.

4.4.2 The maximum of power output

When the current was at steady stage, a polarization curve was also measured to determine what load would produce the maximum power generation. The current density was plotted against voltage and power density at various external resistances (shown in Fig.4.8). The electrochemical property of Mn-MFC was compared with that observed from Fe-MFC. The curves were similar in the low current density region. In Fe-MFC, the maximum power density of 31.02 mW/m^2, which was obtained with current density of 119.42 mA/m^2 and 130Ω, was lower than 60.77mW/m^2 of Mn-MFC, which associated to a current density of 192.38 mA/m^2 at 100Ω. This result shows that under effect of soluble redox mediators the performance of the cell was enhanced more efficient with manganese ion than ferric ion. According to the concept of resistance matching, power transfer is at its most efficient when the load resistant matches the internal resistance of the cell. In other words, internal resistance of two MFCs was approximately a range of

100Ω÷130Ω when the peak power density was generated. This test illustrated that the maximum of power density was signifinicantly improved by effect of Fe^{3+} and Mn^{4+}.

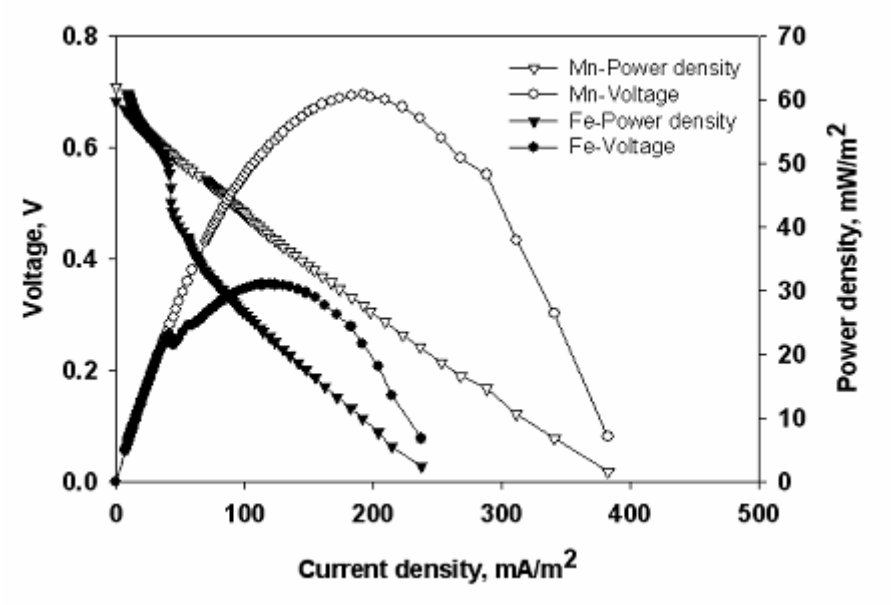


Fig.4.8 Polarization curve for comparison power density and current density between Mn-MFC and Fe-MFC.

According to polarization curve, the higher the current, the greater the voltage drops. In Fig.4.8 shows that voltage of Fe-MFC drop faster than of Mn-MFC. It means Fe-MFC has higher ohmic loss compare with Mn-MFC. In addition, to explain the reason of significant increasing in power output in the presence of ferric and manganese ions, changes in the maximmum value of V_{oc} was plot according to time. From the data in Fig.4.9, it is apparent that both Fe-MFC and Mn-MFC have higher V_{oc} than the control MFC. The higher V_{oc} means lower activation losses.

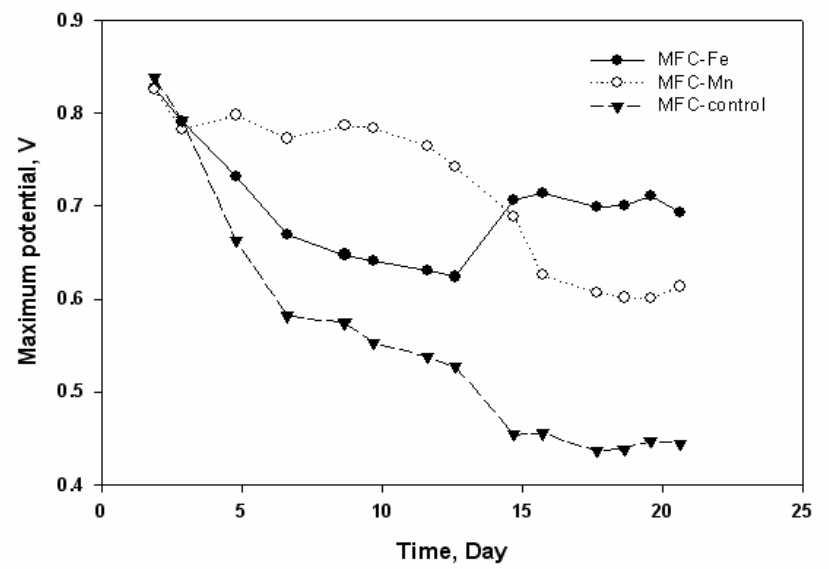


Fig.4.9 Comparision of maximum power output during operation time.

Further analysis showed that microbial activity of microorganism was more efficiency in case of Fe/Mn-MFC (Fig.4.10). The rate of microbial reaction corresponds to the rate of maximum voltage (V_{oc}) which was obtained from Modified Gompertz equation.

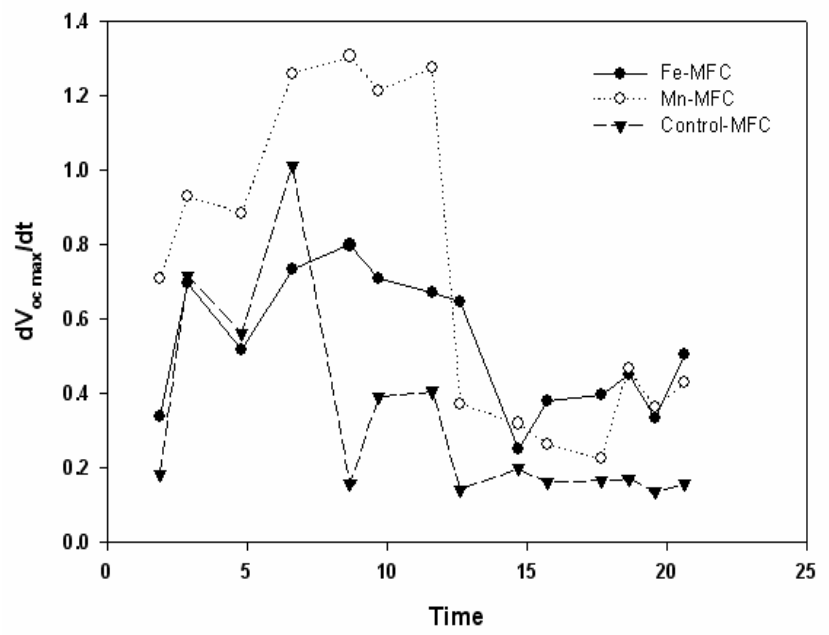
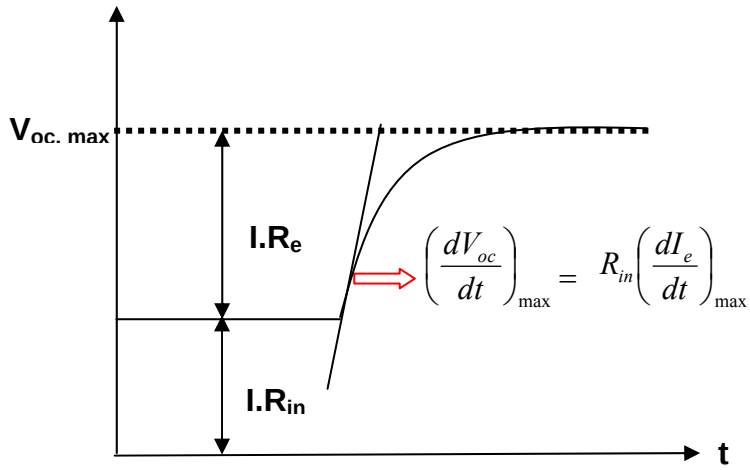


Fig.4.10 The rate of electron production of three MFC



Ordinarily, bacteria can donate electrons to a chemical electron mediator, which in turn transfers the electrons to an electrode, producing electricity. In current research reported that electrons span from outer membrane cell were shuttled to surface carbon electrode without chemical transfer mediator [2]. Therefore, researchers focus how on increasing higher electron transfer rate. Bennetto (1990) demonstrated that microbes could be exploited to produce electricity in an MFC. Park and Zeikus (2000) developed strong comparisons between the two-compartment fuel cell and the single compartment fuel cell. Lovley and Chaudhuri (2003) found the microbe *R. ferrireducens*, a bacterium with the highest electron transfer efficiency (80% of theoretical electron content was transferred to an electrode). Jang et.al (2003) developed a membrane-less and mediator-less microbial fuel cell. In the anolyte, electrons are liberated by degradation of glucose. Some studies demonstrated that MO has a capability of electron transfer process directly. A general route for transferring electrons is that redox proteins (e.g. cytochomes) shuttle electrons directly to electrode surface. Beside that the role of Fe/Mn as nutrients in biochemistry, an important molecule of cytochrome, under anaerobic conditions, soluble ferric ions or manganese ions can also act as electron mediator through redox process. In the absence of oxygen, Fe/Mn oxides are

subjected to reduction to Fe^{2+} and Mn^{2+} by accepting electrons released from microorganism. They are reoxidized back to Fe^{3+}/Mn^{4+} at the electrode surface by biocatalyst such as ion/manganese oxidizing bacteria. Thus, there will be continuous redox cycles of Fe/Mn in anodic compartment. Fig.4.13 describes how electrons were shuttled to electrode in the present of these metal ions. In Fe-MFC, where ferric ion was used similar as a role of electron mediator, it is quickly to transfer electrons from bacterial membrane cell to the anode. There is the same a performance to that of Mn-MFC. For this reason, the current generated in two MFCs are higher and longer term stability of biological activity than of the control. In control MFC, without Fe^{3+}/Mn^{4+} as soluble electron mediator, electrons transfer to the anode is slow. A possible explanation for this might be that Fe/Mn ion has high redox potential to prefer reacting electrochemical reaction to electrobiological reaction (explained in Fig.4.11)

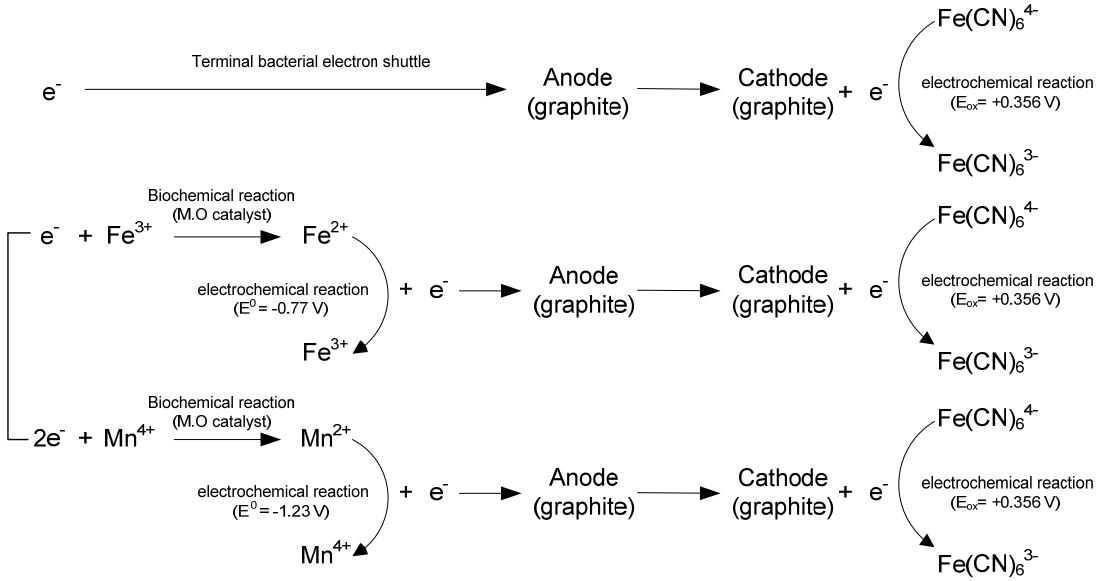


Fig.4.11 Diagram of the electron transfer route from bacteria to terminal electron acceptor

4.4.3 Interal resistance of MFC in the presence of Fe^{3+} and Mn^{4+} ions as solube redox mediators

The internal resistance versus current density for three MFCs is presented in Fig. 4.14. As shown in this figure, the current density of cell could be increased by the decrease in internal resistance. The reduction of internal resistance of control-MFC can enhance a small value of current density whereas Fe-MFC and Mn-MFC obtain a little bit of current density. For example, a maximum current density of 0.13mA/m^2 with 73.5Ω and 0.12mA/m^2 with 66.9Ω are observed for Fe-MFC and Mn-MFC, respectively. So, electron transfer was enhanced by the presence of two ions might cause to decrease the internal resistance of system.

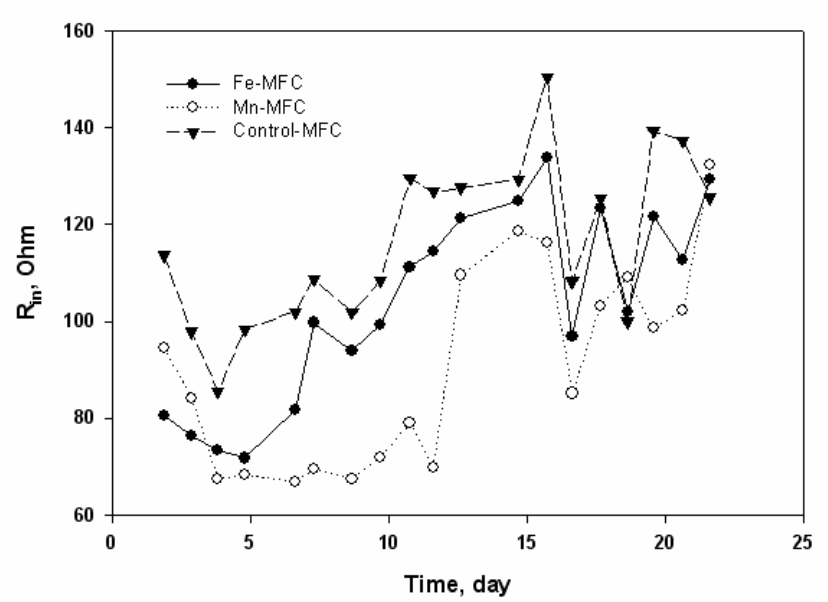


Fig.4.12 Internal resistance of MFC system during operation.

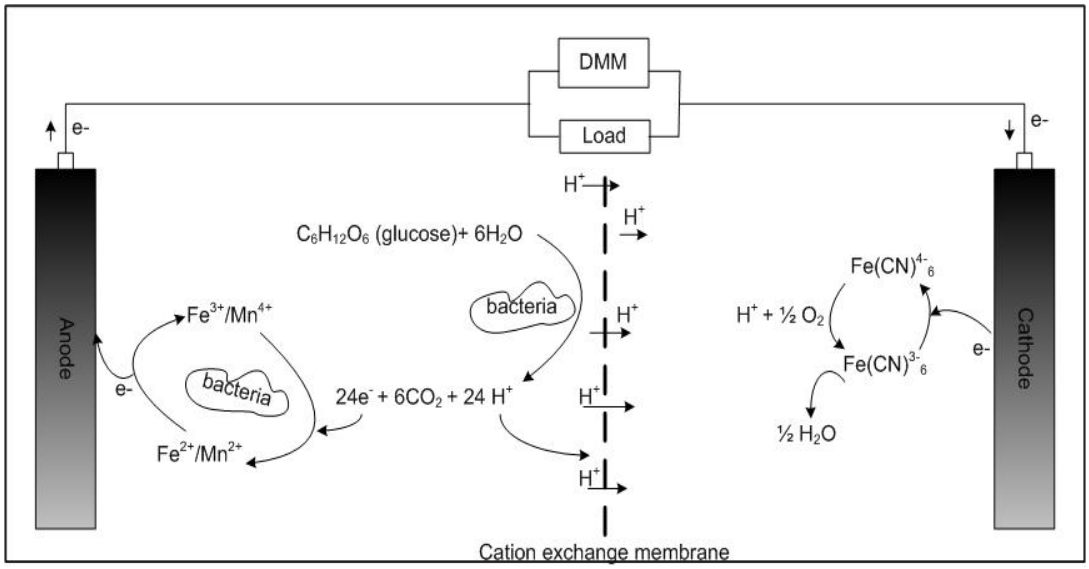


Fig.4.13 Schematic configuration of MFCs. Glucose was oxidized in the anodic compartment and the electron were transferred to electrode via soluble redox mediator (Fe^{3+} or Mn^{4+}) and in cathode compartment hexacyanoferrate was reduced by the electrons derived from the anodic reaction and reduction of electron acceptor in the presence of dissolve oxygen available in the solution.

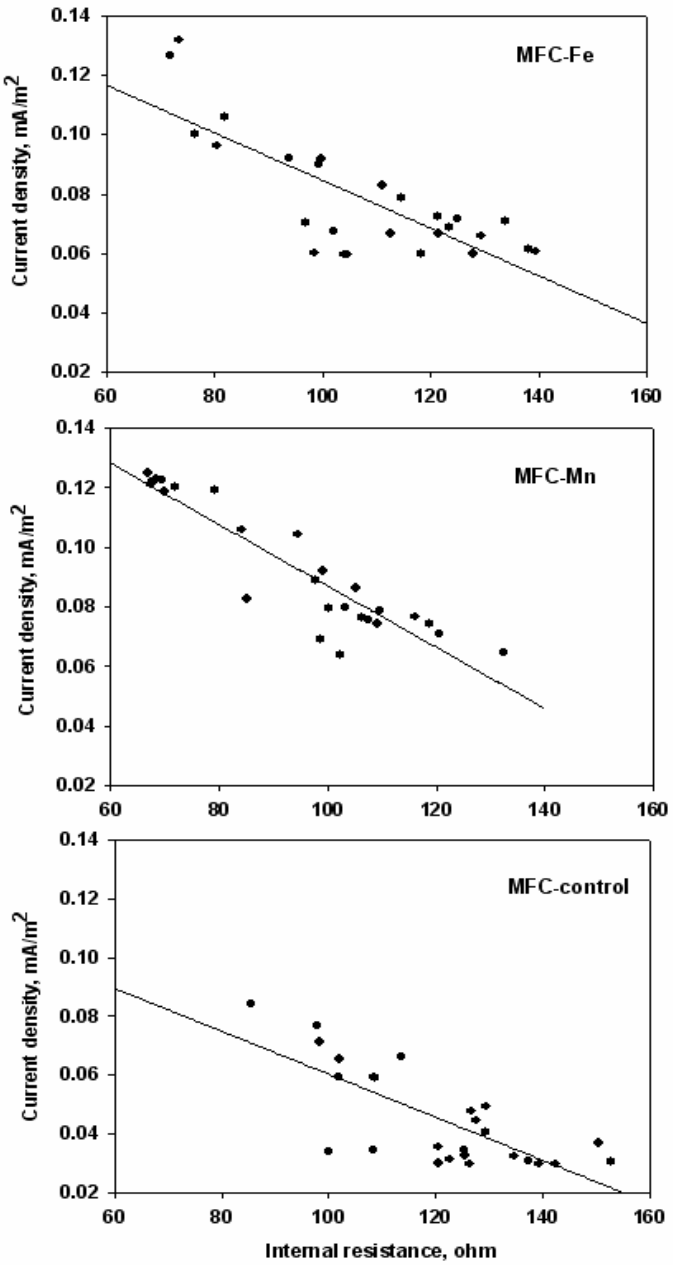


Fig.4.14 Relationship between internal resistance and current density (geometric area of the anode) of three types of MFCs

4.4.4 Power production in the presence of Fe^{3+} / Mn^{4+}

To evaluate the performance of three MFCs, power density and coulomb cumulation are accounted and compared in Fig.4.15. And coulomb efficiency of them is also compared. As Table 4.3 shows, coulomb yield of Mn-MFC was highest and higher three fold than of the control MFC. In case of Fe-MFC, it also shows a clear increase in coulomb efficiency and also has higher double coulomb efficiency than of control-MFC.

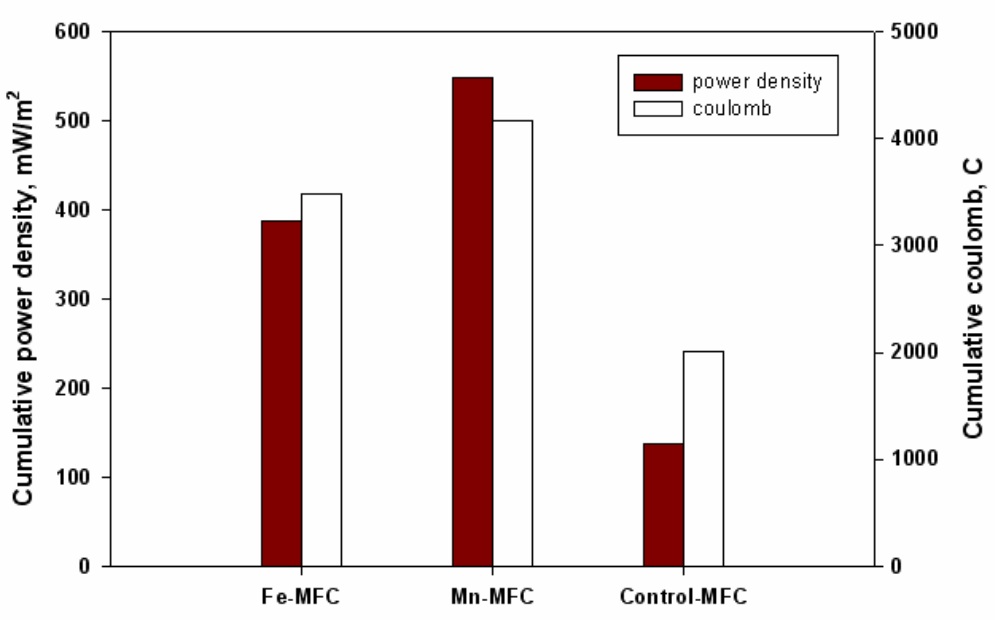


Fig.4.15 Electricity generation with the effect of Fe(III)/Mn(IV) ion as electron carrier were higher than control

As expected, the role of Fe/Mn ion solution in the route of electron transfer in MFC was investigated. In the presence of soluble redox mediators such as ferric and manganic ion, the voltage of the cells increased to higher initial value, steady value and had longer-term steady state in current than the conventional MFC. Cells dying over time were as a result of changes in pH. It causes the power generation drop to low value of potential after a period of time in batch mode. However, in the

presence of Fe or Mn, the rate of electron transferring of mediator to the anode was improved significantly. This result has useful meaning for reference of continuous MFC system.

Table 4.3 Comparison of current density, internal resistance and coulomb yield of three MFCs

MFC	I_d (mA/m^2)	R_{in} (Ω)	CE (%)
Fe	78.37±0.31	107.66±20.06	58.5
Mn	92.86±0.33	94.27±19.33	83.7
Control	58.54±0.28	103.87±13.37	26.8

The following conclusions can be drawn from this topic: (i) the rate of electron transferring to the anode was improved significantly, (ii) higher microbial activity, lower Ohmic losses (iii) power output and efficiency of MFC were enhanced up to 2-3 folds comparing to control MFC. The evidence from this topic suggests that electricity generation could be improved by using of Fe/Mn ions to enhance the rate of electron transfer from bacteria to the surface electrode in anode compartment.

4.5 Effect of glucose concentration on the electricity generation

Various substrate concentrations were supllied to MFCs to test effect of substrate on electricity generation. As Fig.4.16 shows, there is a trend of coulomb efficiency according to substrate concentration. The higher concentration of glucose the smaller yield of electricity production despite higher maximum power density was recorded. It seems possible that the result is due to the greater ability of some

bacteria to metabolize certain by-products obtained from the oxidation of glucose. Another possible explanation for this is that high degree of concentration was inefficiency for favourable growth of cells and cause to the diminution of voltage registered and reduced speed of reaction (according to M.C Potter, 1912). Similarily, in this study, the speed of reaction varies with the amount of substrate present in the medium. However, more research on this topic needs to be undertaken before the association between initial substrate condition and yield of electricity generation is more clearly understood. For example, it's necessary to monitor composition of VFA (volatile fatty acid) and biogas production in order to explain some energy loss in form of these by-products.

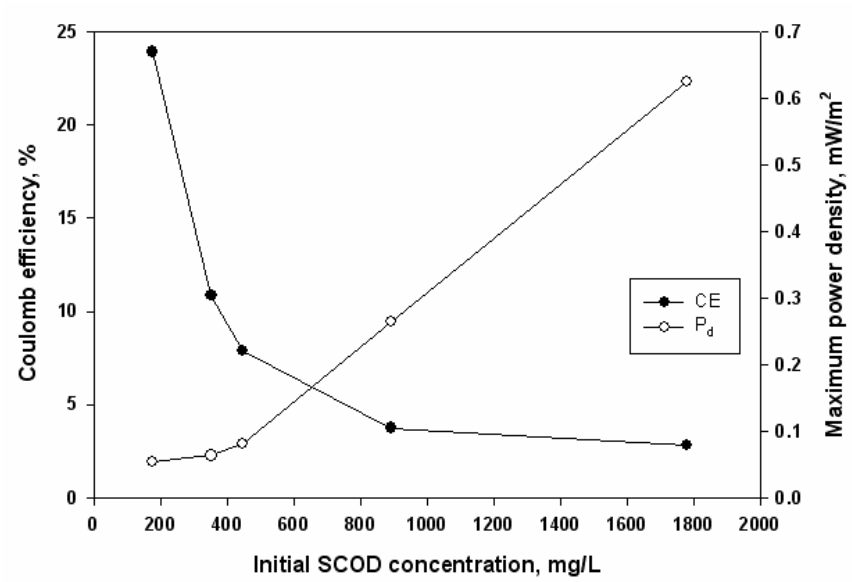


Fig.4.16 Changes in coulomb efficiency and maximum power density as a function of substrate concentration.

4.6 Effect of different inoculum on electricity generation

4.6.1 MFC performance

In this test, three types of inoculum (activate sludge-AS; heat treated sludge-HT and anaerobic sludge-AnS) were used. Electricity generations increased sharply after their short lag phase,as shown in Fig.4.17, and approached their maximum values (0.49V, 0.51V, 0.53V) within 60h, 100h, and 90h following MFC inoculated with activate sludge, heat treated sludge and anaerobic sludge respectively. Among of three MFCs, AS-MFC performance is worse than the others. The AnS-MFC illustrated that electricity generation could maintain longest term. It maintained the voltage at steady state for over 16 days whereas AS-MFC and HT-MFC started to drop down after 10 days.

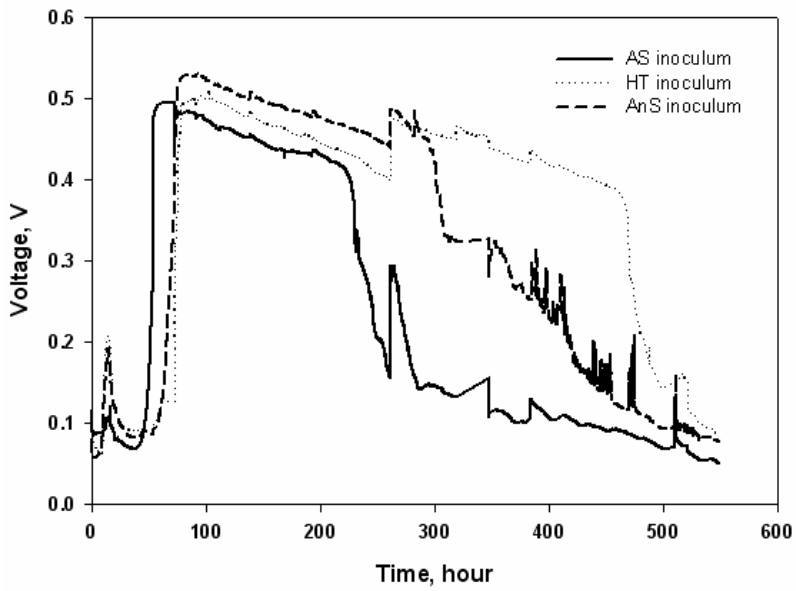


Fig.4.17 MFC performance of different types of inoculum during operation. Solid line, AS- MFC; Dotted line, HT- MFC; Medium dash line, AnS-MFC.

For explaining, value of V_{oc} was simulated using Modified Gompertz model. Then, maximum potential output-$V_{oc,\,max}$ and the maximum rate of electricity production -

$(dV_{oc}/dt)_{max}$ were estimated by fitting the observed cumulative $V_{oc,\ max}$ into equation (described in chapter 2, item 2.3) and plotted in Fig.4.18 and Fig.4.19, respectively. The data shows that all of them have similar value of $V_{oc,\ max}$ at steady- stage. It means their potential of power output were not much different. After that, however, there were great differences in maximum value of potential. $V_{oc,\ max}$ of AS-MFC apparently dropped to lower than of the others. From this data, it can be seen that their microbial activities which represent rate of electricity production depended on type of inoculum. With activated sludge, microorganism might be not high activity. Therefore, the rate of electricity production is slowest among three type of inoculum. Electron transfer from outer membrane of cell to electrode might be not as fast as possible because of some losses on their transfering route or some internal resistance in this system. It is shown that using different types of sludge as inoculum (e.g. AS, HT, AnS) to generate electricity, maximum power density was 63.50mW/m^2 (CE= 46.80 %) with AS-MFC, 67.79mW/m^2 (CE=47.20%) with HT-MFC, and 73.35mW/m^2 (CE=66.36%) with AnS-MFC. (Fig. 4.20)

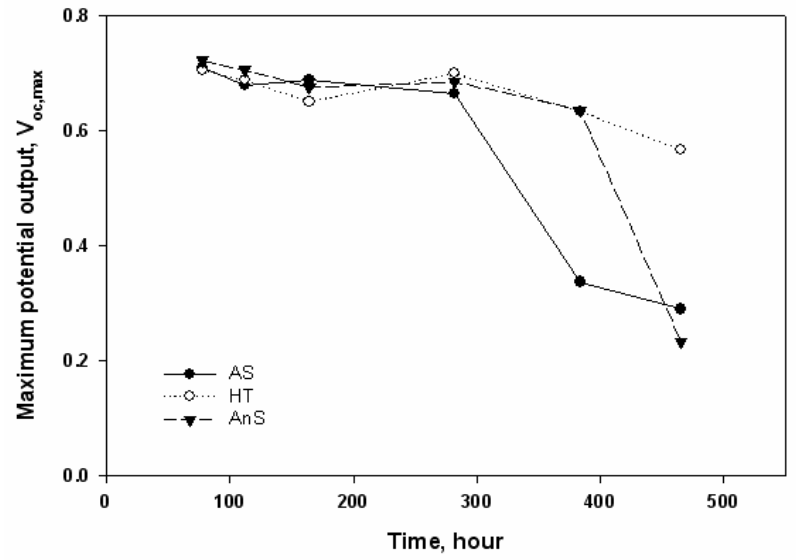


Fig.4.18 Comparison maximum potential output of MFC inoculated with different types of inoculum during operation time (AS, HT and AnS)

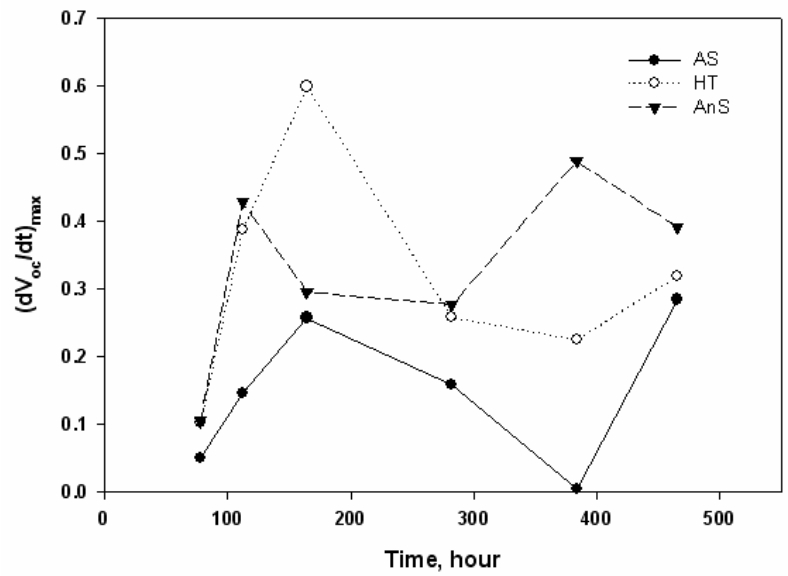


Fig.4.19 Comparison the maximum rate of electron production of MFC inoculated with different types of inoculum.

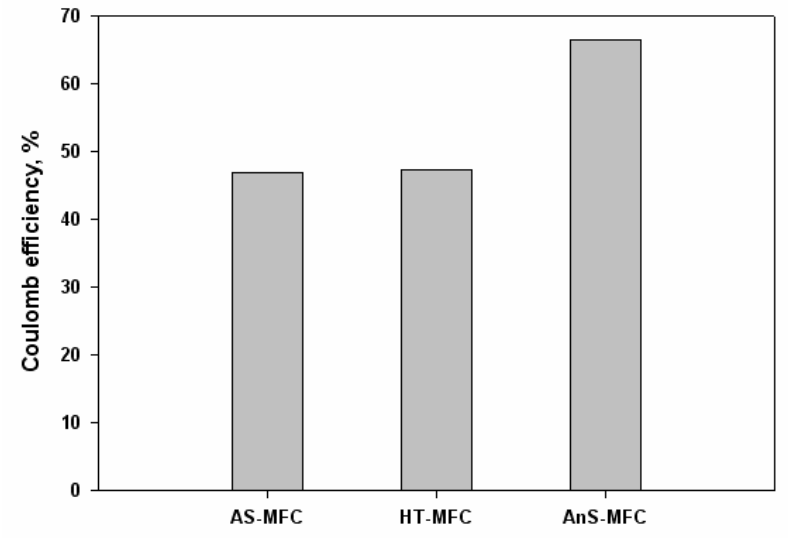


Fig.4.20 Coulomb efficiency for MFCs with different types of inoculum

4.6.2 Internal resistance

Analysis SCC (short circuit current) data, the similar results were obtained at different types of inoculum in three MFCs. Fig. 4.21 shows that electron produced in AnS-MFC was highest follow by HT-MFC, thus, their internal resistance also lower than of AS-MFC (Fig.4.22). This demonstration enhanced the reason of greater power output in AnS-MFC as decrised above.

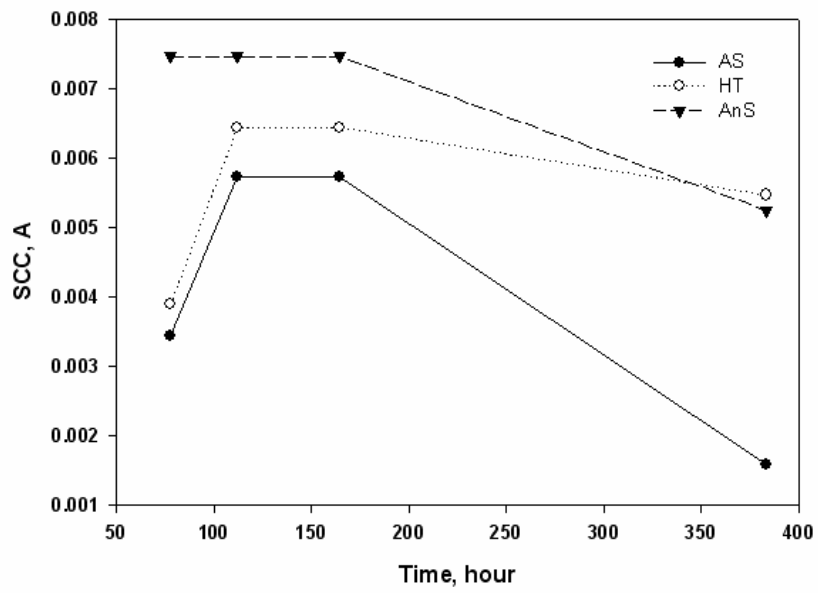


Fig.4.21 Comparison short circuit current for MFCs with different types of inoculum.

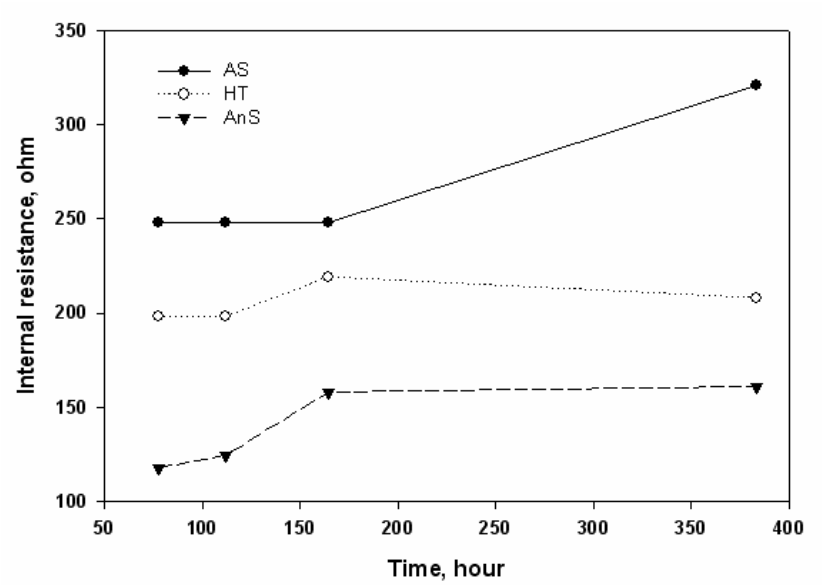


Fig.4.22 Comparison internal resistance of MFC inoculated with different types of inoculum.

4.6.3 Observed biogas production

It is interesting to note here that in three cases, hydrogen gas produced in anode compartment significantly. As shown in Fig.4.23, cumulative hydrogen productions increased gradually and were proportion to electricity generation in three MFCs. The differences in the observed maximum hydrogen productions might be attributed to the variation in the dominant species of hydrogen producing bacteria (anaerobic condition, heat treatment condition). For example, anaerobes such as Clostridium available in anaerobic sludge produce hydrogen, but are incapable of completely utilizing energy or decomposing organic substances.

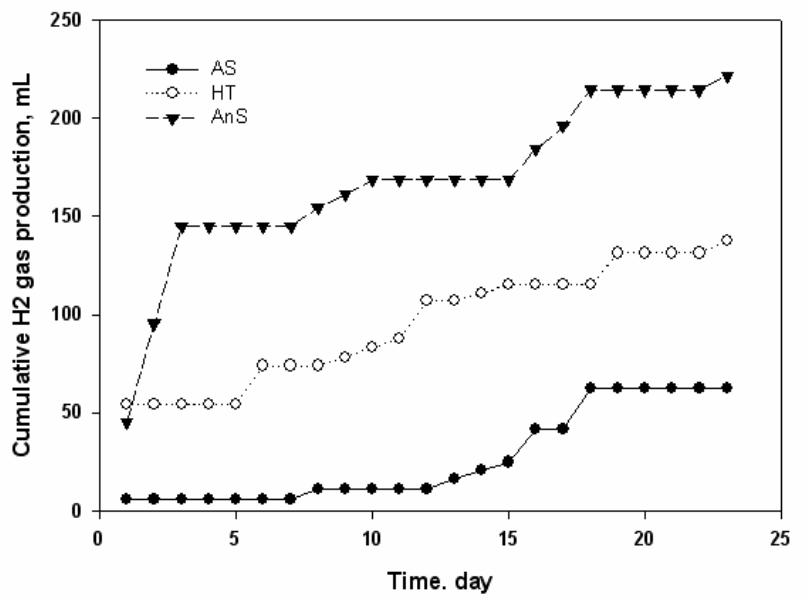


Fig.4.23 Cumulative hydrogen production of MFC inoculated with different types of inoculum.

In principle, microorganism captures energy released from oxidation- reduction reactions and uses an electron donor substrate for synthesis (f^0_s) and generates energy (f^0_e). The energy released per electron transfer:

$$1 = f^{\theta}_{e} + f^{\theta}_{s} \; and \; f^{\theta}_{e} = f^{\theta}_{ee} + f^{\theta}_{eo} \;\rightarrow\; 1 = f^{\theta}_{s} + f^{\theta}_{ee} + f^{\theta}_{eo}$$

Where f^0_e is energy using for cell maintaince, f^{θ}_{s} is enegry using for microbial synthesis and growth. Energy for cell maintain is comprised two parts, f^{θ}_{ee} and f^{θ}_{eo}. Base on degradation of glucose, percentage of energy transfer to electric energy and hydrogen gas was calculated. In this MFC system, glucose is used as electron donor source and engery released from oxidation of substrate under anabolism and ultimately generates CO_2. Electrons are donated as follows:

$$\frac{1}{24} C_6 H_{12} O_6 + \frac{1}{4} H_2 O = \frac{1}{4} CO_2 + H^+ + e^-$$

$$\Delta G^{O'} = -41.35\, kJ$$

However, there are several steps involving above reaction, and H_2 is possible formed at early steps or during acetogenic fermenters. Free energy is provided from H_2 reduction as below reaction:

$$H^+ + e^- = \frac{1}{2} H_2$$

$$\Delta G^{O'} = 39.87\, kJ$$

Therefore, it is possible that amount of electrons released from degration of substrate process transfer to terminal electron acceptor (graphite) to generate current or directly to form hydrogen gas. In this study, hydrogen gas is the main conmponents of the biogas and so methane and carbon dioxide contents could be neglected. Table 4.4 shows parameters obtained from Modified Compertz model.

Table 4.4 Summary the maximum rate of hydrogen production, potential of hydrogen production and lag phase time of three MFCs

MFC	R_m	H_{pu}	λ (hr)
AS	5.68	89.11	12.19
HT	5.65	164.95	0
AnS	9.34	233.05	0

CHAPTER 5
CONCLUSIONS AND FURTHER STUDY

5.1 Conclusions

In the experiment of VSS effect, the results showed that internal resistance of the MFC system could be reduced by the control of VSS concentration. In addition to, role of starved bio-film and suspended MO on electrical output in MFC were also investigated. In the available presence of them, electrical energy could be increased to higher value of power output than before. The longer starvation time the shorter steady stage. However, starvation has a negligible effect on electricity generation of MFC. Starvation effect on power output by biofilm doesn't get higher yield than no starvation.

A reason of all MFCs decreased their potential after a period of time is almost ferricyanide in cathode converted to ferrocyanide. It causes protons transfer through PEM to cathode can not combine with electrons come from ex-wire completely.

The presence of Fe^{3+} and Mn^{4+} under form of soluble salt in anolyte solution showed the significantly improvement of power output. A maximum current of 2.06-1.92 mA and power density of 69.02-61.84 mW/m^2 were obtained from batch 1 and batch 2, respectively. The coulbomb efficiency of batch 1 and batch 2 which had ferric ion and manganese ions were enhanced up to 2-3 folds comparing to reference batch. The result showed that electricity generation of MFC could be enhanced by electrochemical redox of Fe/Mn ions. In the presence of ferric/manganese ion in anode compartment, the rate of electron transferring to the anode was improved significantly. This means higher microbial activity and lower

Ohmic losses.

One of the more significant findings to emergy from this study is that hydrogen gas could be produced directly from anode compartment beside electricity. MFC inoculated with anaerobic sludge gave the best performance (66.36 CE %) and highest yield of hydrogen production (46.91%). Following by MFC inoculated with heat treated sludge (47.20 CE % and 17.80 % yield of hydrogen production). MFC inoculated by activated sludge archieved only 46.80% of coulomb efficiency and yield of 12.65% hydrogen production. However, more research on this topic needs to be undertaken.

The information from these batch modes is valuable for application of a continuous reactor MFC.

5.2 Further study

To reduce the overpotential losses of MFC system, further research should be done some more factors to investigate the influent of them on electricity generation. Thus, future studies on the current topics are therefore recommended:

- For deeply understanding Fe^{3+} and Mn^{4+} effect on electron transfer, an optimal concentration of these ions will be examined
- It’s necessary to analyze composition of VFA (volatile fatty acid) and biogas production to determine metabolism pathway of bacteria.
- Electrode reaction of bacteria cells also need to be test by cyclic voltammogram for explanation of electrochemical property of MFC
- Some environmental factors should be tested such as pH, ORP, HRT, OLR, mixing rate, types of carbon source etc…
- Continuous reactor will be set up with optimal parameters which determined by batch tests from this study.

요 약

미생물 연료전지(Microbial Fuel Cell)는 유기물에 저장된 생화학적 에너지를 전기화학적으로 활성을 가진 미생물을 이용하여 전기 에너지로 변환시키는 장치이다. 본 MFC연구에서는 미생물의 종류(부유미생물, 생물막에 흡착된 미생물), 기질결핍의 영향, 전자전달 효율을 높이기 위한 산화환원 매개체의 사용 그리고 미생물의 활성도를 알아보기 위해 여러 가지 종류의 미생물을 식종해 보았고, MFC시스템의 내부저항 및 전위손실에 관한 연구를 수행하였다. 본 연구의 모든 회분식 실험에서는 두개의 반응조로 구성된 'H'형 모양의 반응조를 사용하였다.

MFC 시스템의 전기 생산 능력을 향상시키기 위해서 본 연구에서는 부유미생물과 생물막에 흡착된 미생물의 영향을 연구했고, 전기적으로 전위차가 다른 혐기성 및 호기성 미생물과 열처리된 미생물을 식종함으로써 미생물 식종에 따른 영향을 연구하였다. 음극에 식종된 다양한 식종 미생물에 대하여 수소생산의 가능성을 알아보았고 그리고 음극 반응조에 Fe3+/Mn4+ 이온과 같은 산화환원 매개체를 이용하여 전자전달에 영향을 주는 요소들에 대해 연구하였다.

부유미생물과 생물막에 흡착된 미생물의 영향을 알아보기 위해 기질결핍 상태와 기질이 충분한 상태의 조건아래서 실험하였다. 실험결과에서 보면 부유미생물을 이용한 실험에서 MFC 시스템의 내부저항이 감소하였다. 그리고 생물막에 흡착된 미생물을 이용한 실험이 전극에 미생물이 흡착되어 있지 않은 것보다 전기생산량이 더 높게 나타났다. 장시간의 기질결핍 조건에서는 정상상태가 유지되는 기간이 더 짧았다. 그러나 본 연구에서 기질결핍 조건은 MFC 시스템의 전기 생산에는 효율적이지 않았다. 생물막에 흡착된 미생물을 이용한 기질결핍의 조건에서 전기생산량은 기질이 충분한 상태보다 높지 않았다.

철 이온과 망간이온을 이용한 실험에서 음극 반응조 미생물의 전자전달은 크게 향상되었다. Fe-MFC 와 Mn-MFC의 최대 전류값은 각각 2.06 ~1.92mA, 전력밀도는 69.02 ~ 61.84 mW/m2 를 나타내었다. Fe-MFC 와 Mn-MFC의 쿨롱 효율은 Control MFC보다 2-3배 높았다.

수소 생산 반응조의 최대 전압과 최대 수소 생산량은 Modified Gompertz방정식을 이용하여 만든 곡선식으로 분석하였다. Modified Gompertz방정식에 의하면 혐기성 슬러지를 식종한 MFC의 수소생산량이 46.91%로 가장 놓았고, 쿨롱효율이 66.36%로 제일 높은 효율을 나타냈다. 두 번째는 열처리 슬러지를 식종한 MFC로서 수소생산량 17.80%, 쿨롱 효율 47.20%를 나타냈다. 마지막 호기성 슬러지를 식종한 MFC는 수소 생산량 12.65%, 쿨롱 효율 46.80%로서 수소 생산량과 쿨롱 효율이 가장 낮았다.

본 연구의 결과를 통해서 미생물 연료전지의 효율을 향상 시킬 두 가지 요인을 알 수 있었다. 첫 번째로 철 이온, 망간 이온 같은 산화환원 매개체를 이용하여 음극에서 양극으로의 전자 이동속도를 향상시킬 수 있다. 두 번째는 미생물연료전지의 음극 반응조에서 수소를 생산 할 수 있다. 본 연구를 토대로 연속식 미생물 연료전지 반응조를 위한 중요한 실험 인자들을 찾을 수 있었다.

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