ELECTRICITY GENERATION FROM DEWATERED SLUDGE USING MEMBRANE-LESS MICROBIAL FUEL CELL

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UNIVERSITI SAINS MALAYSIA

2018

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by

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Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

July 2018

AKNOWLEDGEMENTS

Alhamdulillah, all praises to Allah SWT, the most gracious and the most merciful, and may peace blessing of Allah be upon His messenger, Prophet Muhammad pbuh. First and foremost, I would like to express my gratitude to His greatness, with whose indulgence has given me the strength and convenience to complete this research successfully. I would like express my deep and sincere gratitude to my late supervisor Allahyarhamah Prof. Dr. Mashitah Mat Don, who always believed in my ability to be successful in the academic arena. You are gone but your belief in me has made this journey possible. Although no longer with us, continues to inspire by her example and dedication to the students she served over the course of her career.

I owe my most sincere gratitude to Assoc. Prof. Dr. Vel Murugan Vadivelu, my PhD supervisor, with his enthusiasm, his inspiration, and his great efforts to explain things clearly and simple for me. Throughout my thesis writing period, he provided encouragement sound advice, good teaching and lots of good ideas. There is huge transition for me from a lonely PhD candidate without supervisor to a candidate with full of new motivation and hopes. I would have been lost without him. I am deeply grateful to Dr. Suzylawati Ismail and Assoc. Prof. Soib Taib for their advices and friendly help. Their extensive discussions around my work and interesting explorations in operations have been very helpful for this study. In sincerely thank to them for the support they extend to me.

I would like to pay homage to the Dean of School of Chemical Engineering, USM, Professor Dr. Azlina Harun @ Kamaruddin for her support and help concerning my postgraduate affairs. I am also very much thankful to all staff and technicians of School of Chemical Engineering for their co-operation and warmest helping hand for the past few years.

I am deeply grateful to my parents, Mr. Mohd Zaini Makhtar bin Che Kasim and Mrs. Jamaliah binti Abd Halim for their endless love and blessing for me to pursue my Ph.D degree. I am also grateful to my dear and beloved wife, Nor'Izzah binti Zainuddin, whose love and affection and unimaginable support is boundless. I welcome my little son, Muhammad Daniyyal Rizqi bin Muaz, to this world and share our love and affection, which is a beautiful part in my life. Apart of that thanks to my sister Salwa, Hafizah, Nurul Shukriah and my lovely brother Zaid for their longstanding support from the beginning of my life till today.

I would also like to thank Rasyid Ruslan who was a best colleague from the lab for his constant support and I enjoyed working with him. His constructive and excellent advice helped me bring my thesis in a good shape. Words fail me to express my-heart gratitude to all my fellow friends and colleagues, who were always been great support in all my struggles and frustrations along my study. We could always share problems, excitement and supports among each other. Last but not least, Finally, I appreciate the financial support provided by Ministry of Education, Malaysia (MyPhD) and USM (RUI) Grant (1001/PJKIMIA/814267) throughout my postgraduate study.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spetrophotometer
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AQDS	Anthraquinone disulphonate
AHQDS	Anthrahydroquinone disulphonate
CCD	Central composite design
CFU	Colony-forming unit
CO_2	Carbon monoxide
COD	Chemical oxygen demand
FADH	Flavin adenine dinucleotide hydrate
FAD	Flavin adenine dinucleotide
ML-MFC	Membrane-less microbial fuel cell
LED	Light emitting diode
NAD^+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide hydrate
ODW	oven-dry sample weight
OFAT	One-factor-at-a-time
PEM	Proton exchange membrane
RMSE	Root Mean Square Error
RSM	Response surface method
SHE	Standard hydrogen electrode
VS	Volatile solid

LIST OF SYMBOLS

X_m	Maximum biomass concentration
X_c	Critical biomass concentration
μ_m	Maximum specific growth
μ	Specific growth rate
X_o	Initial biomass concentration
X	Biomass concentration
t	Time
t_c	Critical time
δ	Non-growth-associated substrate
Υ	Growth-associated substrate
DF	Degree of freedom
P	Product
S_o	Initial substrate (COD initial)
S	Substrate (COD)
n	Constant
Y	Response
H^+	Proton
e^-	Electron
R^2	correlation of determination

PENJANAAN ELEKTRIK DARIPADA SEL BAHAN BAKAR MIKROB TANPA MEMBRAN

ABSTRAK

Sel bahan bakar mikrob tanpa membran (ML-MFC) merupakan inovasi teknologi tenaga boleh diperbaharui yang mampu menjadi tenaga alternatif bagi mengatasi krisis tenaga dunia. Enapcemar ternyah air daripada tiga loji rawatan kumbahan yang berbeza (A – IWK Kerian, B – IWK Butterworth and C – IWK Juru) digunakan sebagai substrat di dalam sel bahan bakar tanpa membran (ML-MFC). ML-MFC beroperasi secara elektrokimia bersama-sama bakteria elektrogenik (EB) yang bertindak sebagai pemangkin biologi bagi menghasilkan tenaga elektrik. Dapatan daripada ujian awal, enapcemar A mempamerkan prestasi yang baik berbanding enapcemar yang lain daripada segi komposisi nutrient, nilai COD dan kuasa yang terhasil. Seterusnya prestasi ML-MFC dinilai menggunakan kaedah satu factor pada satu masa (OFAT) kemudian diteruskan ke kaedah permukaan sambutan (RSM) melalui reka bentuk komposit pusat menggunakan model kuadratik. Dalam kajian awalan OFAT, penjanaan voltan (852.7 mV) dan penyingkiran COD (149.2 mg/L) tertinggi dicatatkan apabila pH 6.0, jarak elektrod (ED) 3 cm, kandungan lembapan (MC) 30 % (v/w) dan suhu 35 $^{\circ}$C. Selepas inkubasi ML-MFC menggunakan keadaan optima yang dicadangkan oleh RSM (ED 3 cm, MC 32 % v/w, temperature 38 $^{\circ}$C), voltan (927.7 mV) dan penyingkiran COD (170.8 mg/L) telah berjaya ditingkatkan kepada masing-masing 8.79 % dan 14.47 %. Ini menunjukkan pengoptimuman menggunakan kaedah RSM memberikan keputusan yang lebih baik daripada kaedah OFAT dan ketumpatan kuasa maksimum (41.31 mW/m^2) telah dicatatkan.

Pemerhatian mikroskop electron pengimbas (SEM) telah mendedahkan pembentukan biofilm EB di permukaan anod. Analisis filogenetik EB membuktikan kehadiran spesies *Pseudomonas* dan *Bacillus subtilis* Kajian ini juga menunjukkan model pertumbuhan kinetik tidak berstruktur, Logistik, menggambarkan dengan baik perilaku pertumbuhan EB di dalam ML-MFC dengan catatan R^2 yang tinggi (0.991) dan RMSE yang rendah (0.189). Manakala model Leudeking-Piret-like bagi penyingkiran COD juga mencatat keputusan yang baik dengan nilai R^2 dicatat pada 0.971 dan mempunyai nilai RMSE yang rendah iaitu 0.203. Data eksperimen menunjukkan bahawa model Logistik dan Leudeking-Piret-like dapat menggambarkan pertumbuhan EB dan penyingkiran COD dalam ML-MFC. Analisis ketidakpastian parametrik terhadap penyingkiran COD kemudiannya dinilai mengunakan simulasi Monte Carlo (pembolehubah stokastik) untuk menentukan pengagihan kebarangkalian disebabkan oleh aliran turun naik dan variasi parameter model kinetik. Keputusan menunjukkan bahawa berdasarkan 100,000 sampel yang diuji, penyingkiran substrat (*S*) adalah dari 179.23 – 191.13 mg/L. Analisis sensitiviti juga dilakukan untuk menilai kesan setiap parameter kinetik kepada prestasi ML-MFC. Didapati prestasi ML-MFC sangat bergantung kepada pertumbuhan EB yang hadir di dalam ML-MFC.

ELECTRICITY GENERATION FROM DEWATERED SLDUGE USING MEMBRANE-LESS MICROBIAL FUEL CELL

ABSTRACT

The membrane-less microbial fuel cell (ML-MFC) is an innovative renewable energy technology that becomes the alternative energy to overcome the global energy crisis. The ML-MFC operated electrochemically incorporate electrogenic bacteria (EB) acted as a biocatalyst in order to produce electricity. Dewatered sludge from three different wastewater treatment plant (A – IWK Kerian, B – IWK Butterworth and C – IWK Juru) were used as substrate in the ML-MFC. From the preliminary test sludge A showed a better performance compared to the others in term of nutrient composition, COD value and power generation. Then performance of the ML-MFC using sludge A was evaluated using one-factor-at-a-time (OFAT) method followed by response surface methodology (RSM) via Central Composite Design using a quadratic model. In the preliminary OFAT study, the highest voltage generation (852.7 mV) and COD removal (149.2 mg/L) were obtained when the pH 6.0, electrode distance (ED) 3 cm, moisture content (MC) 30 % (v/w), and temperature 35 $^{\circ}$C. After incubation of the ML-MFC using optimum conditions suggested by the RSM (ED 3 cm, MC 32 % v/w, temperature 38 $^{\circ}$C) the voltage (927.7 mV) and COD removal (170.8 mg/L) were successfully increased about 8.79 % and 14.47 %, respectively. This showed that optimization using RSM gave better results than the OFAT method and the maximum power density (41.31 mW/m^2) was recorded. The scanning electron microscope (SEM) observation revealed the EB biofilm formation

at the anode surface. The phylogenetic analysis of EB proved the presence of *Pseudomonas* and *Bacillus subtilis* species in the biofilm which actively boosted the electron transfer. The study also showed unstructured kinetic growth model, Logistic, describing well the growth behaviour of EB in the ML-MFC with high R^2 value (0.991) and low RMSE (0.189). While the Leudeking-Piret like model for COD removal also performed well with R^2 values recorded was 0.971 and had low RMSE value which was 0.203. The experimental data show that the Logistic and Leudeking-Piret-like model could best describe the growth of EB and COD removal in the ML-MFC. The parametric uncertainty analysis on COD removal was then assessed using the Monte Carlo simulation (stochastic variable) to determine probability distributions due to fluctuation and variation of kinetic model parameters. Result showed that based on 100,000 samples tested, the substrate removal (*S*) was ranged from 179.23 – 191.13 mg/L. Sensitivity analysis was also done to evaluate the impact of each kinetic parameter on the ML-MFC performance. It was found that ML-MFC performance highly depends on growth of EB present in the ML-MFC.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

The lack of access to clean electricity in developing nations has given importance to the development of low-cost, widely applicable energy technologies. As reported by Sovacool et al. (2012), about 1.4 billion people around the world lacked of electricity and almost 2.7 billion still depending on the traditional method of using biomass. Most of them are living in Sub-Saharan, Africa, India and other developing Asian countries (Branker et al., 2011; Sovacool, 2012). It was predicted that this problem would continue steadily until 2030. Table 1.1 shows the number of people without access to electricity on the year 2009 and the prediction for 2020. Sub-Saharan Africa has become the greatest challenging region where only 31 % of the population has access to electricity, which is the lowest level in the world (Sovacool et al., 2012).

According to Leo–Moggie (2001), energy source around the world mostly depends on coal, oil, gas, nuclear, hydropower, and other renewable energy such as solar and biomass (Table 1.2). There are two major problems arised using the conventional non-renewable energy such as fossil fuel (oil and coal) and natural gas. Firstly, the nature of non-renewable energy which is depleting, and secondly there is a significant contribution for the emission of greenhouse gas when the combustion of non-renewable energy such as coal and natural gas took place. Thus lead to the issue of climate change.

The government of Malaysia has formulated various energy-related policies in order to guarantee the long-term reliability and security of energy supply for sustainable social economic development in the country. Among them are National Energy Policies 1979, National Depletion Policy 1980 and Fuel Diversification Policy 1981 and 1999 (Oh et al., 2010).

Table 1.1 Number of people without access to electricity on year 2009 and the prediction on year 2020 (Sovacool et al., 2012)

Countries	**Number of people lacking access to electricity (million)**	**Prediction (million)**
	2009	**2020**
Africa	587	644
Sub-Saharan Africa	585	640
Developing Asia	799	650
China	8	2
India	404	342
Other Asia	387	307
Latin America	31	16
Developing countries	1438	1350
World	1441	1352

Table 1.2 World energy demand by source (Leo-Moggie, 2001)

Energy source	**1980**	**2000**	**2008**	**2020**
Coal	1792	2292	3286	4124
Oil	3107	3655	4320	4654
Gas	1234	2085	2586	3046
Nuclear	186	676	723	920
Hydropower	148	225	276	389
Biomass and waste	749	1031	1194	1436
Other renewable	12	55	82	196
Total world	7228	10018	12467	14765

The increase concern over pollution, resource depletion, and climate change implications of continuing use of conventional fossil and nuclear fuels has prompted a growing interest in renewable energy sources. The energy policies were made to enhance for the sustainable energy. It is estimated that by 2030 about 15-20% of the energy needs will be met by renewable energy (Bilgen et al., 2004). Therefore, there is a strict need for development of new technologies that can make renewable resources accessible to supply this increasing demand. These technologies need to be developed to conserve non-renewable energy reserves and at the same time to make more sustainable technologies in the next decade (Branker et al., 2011; Rahman and Lee, 2006). Reliance on renewable energy is growing; technologies such as solar, wind and biomass energy play an increasingly important role in meeting the energy future (Banos et al., 2011). Microbial fuel cells (MFCs), which convert biochemical energy consisted in the substrate to electrical energy, can be part of it. MFCs can be used in energy production based dewatered sludge biomass (Kiely et al., 2011; Logan and Rabaey, 2012; Nealson, 2006). MFC created a big impact on the community, government and environment. However, an exploration of material and resources that are cheap to be used in MFC to produce electricity is urgently needed (Nealson, 2006).

1.2 Problem Statements

There is a huge energy reserve in the dewatered sludge without being recognized. They are in the form of biodegradable organic matter and the energy could be recovered (Fischer et al., 2011; Nimje et al., 2012). It was reported a conventional wastewater treatment plant in Toronto, Canada, which contained energy about 9.3 times more than the energy used to treat the wastewater (Shizas and Bagley, 2004). While the study by Logan and Rabaey (2012) stated that the processing wastewater for domestic, animal and food approximately consist of 17 GW energy. This amount was equivalent to the energy needed to supply for the whole water infrastructure in the U.S. It is a promising energy and if the energy managed to be recovered the treatment plant could be run using its own energy supply (Kim et al., 2007). Dewatered sludges generated daily from the wastewater treatment plant were analyzed for it capabilities to support growth of the electrogenic bacteria (EB) for the electricity generation (Rao, 2010; Wachenheim et al., 2003). Dewatered sludge can be used as value added substrate instead of polluting the environment.

Approximately 4.2 million cubic meters of sludge is produced annually and it is also estimated that Indah Water Konsortium (IWK) will be producing 7 million cubic meters of sludge by the year 2020 (Alam et al., 2003; Kadir and Velayutham, 1999) and make it the most favorable substrate for bioconversion as they are renewable and abundantly available (Fischer et al., 2011). The efficiency and economic viability of converting sludge to bioenergy depend on the characteristic and components in it (Wang et al., 2013). By harnessing the energy from sludge, the waste industries can become more efficient both financially and environmentally. Previously, a large number of substrates have been explored as feed for MFC such as various kinds of artificial and real wastewater and lignocellulosic biomass in MFCs (He et al., 2015). Zuo and his co-workers utilized residual products from steam-explosion of stover (e.g. hydrolysates or solids) or, alternatively, crushed the stover into a powder prior to use (Zuo et al., 2006). These processes made lignocellulosic biomass more amenable to biodegradation, but also resulted in a process that was less sustainable and amenable to scale-up (Pant et al., 2010).

Typical MFC's configuration requires anode/cathode electrodes, anodic/cathodic chambers, proton exchanges membrane (PEM) and electrode catalyst. These requirements are high in cost especially the cost to fabricate the chambers in order to separate the oxic (cathodic) and anoxic (anode) environment (Li et al., 2014; Logan and Regan, 2006). Besides that, the expense for aeration supply system plus the electricity needed to power the MFC make it not economical. Furthermore, the presence of PEM (typically Nafion) has boost up the overall cost of MFC. The membrane helps to transmit H^+ to complete the electrical circuit in the MFC. MFC faced several problems involving proton transfer efficiency of PEM due to biofouling and oxygen leakage (Kim et al., 2005; Kim et al., 2007). Usage of catalyst is sharing the same obstacle during the implementation of MFC. High cost for the catalysts such as MnO_2, Fe^{3+} and Pt has brought the researchers to use biocatalyst as the alternative way (Kiely et al., 2011). So the alternative solution is needed to overcome the problems. With this view, the feasible, low capital cost, simple configuration and economical MFC is needed.

Despite the numerous studies that have been carried out on electricity generation and stabilization of sludge, there was less reported research on MFC using dewatered sludge as the substrate. Furthermore, there was no systematic information about the influence of operational conditions on the performance of the MFC. Electricity generation and stabilization of dewatered sludge (COD removal) in membrane-less (ML-MFC) can be influenced by both physical and chemical parameters. In this regard, the study need to determine the operational conditions which the electricity generating and COD removal are able to demonstrate the desirable performance and optimized its operations.

The proximate composition of the tested dewatered sludge was also analyzed. The dewatered sludge is heterogeneous in properties and varies in characteristics (Kadir and Velayutham, 1999); consequently, MFC performance may vary (Pant et al., 2010). By accounting for uncertainty in utilization of heterogeneous substrate feasibility studies, it is expected that an efficient performance of ML-MFC can be carried out (Beekmann and Derognat, 2003; Doubilet et al., 1984). There is a need for uncertainty analysis in order to create a more realistic picture of the how much COD would remove and the literature review of this approach does not available yet.

1.3 Research objectives

The main goal of this research is to investigate the performance of ML-MFC using dewatered sludge as the substrate. In view of such potential, this study was carried out based on following objectives:

i) To determine the proximate composition of dewatered sludge from different type of wastewater treatment plant (IWK Kerian, IWK Juru, and IWK Butterworth).

ii) To optimize the operating condition (pH, electrodes distance, moisture content and temperature) in a batch ML-MFC for generation of electricity and COD removal using OFAT and statistical tool RSM methods.

iii) To validate the kinetic models for the growth of the electrogenic bacteria and COD removal in the ML-MFC.

iv) To assess the COD removal using Monte Carlo simulation and sensitivity analysis.

v) To assess the electricity generation and ML-MFC application using series circuit.

1.4 Scope of study

In view of the demand for ML-MFC for renewable energy, this study presents an investigation on dewatered sludge collected from three different wastewater treatment plants (IWK Kerian, IWK Juru, and IWK Butterworth) into the performance of ML-MFC, focusing on different parameter conditions. The dewatered sludge was used as the main substrate for two purposes: 1) as the nutrient-rich anodic and 2) as a pseudo membrane to separate anode and cathode electrode. To further reduce the cost of the MFC operation, an air-cathode MFC using passive air supply was constructed in which one side of the cathode electrode was attached to the moist sludge, while the other side exposed to the air. The two approaches (membrane-less and air cathode technique) could make this MFC affordable to construct and also reduce the cost of operation.

A comprehensive approach has been taken by conducting the experimental and kinetic modelling work. The experiments were conducted in a batch mode by varying parameters which include the effect of pH, electrode distance, moisture content and temperature using OFAT method and RSM via CCD to obtain the optimum condition. The dewatered sludge consisted of mixed culture of electrogenic bacteria (EB), thus enhance the generation of electricity in the ML-MFC. The SEM observation and phylogenetic analysis were done to prove the presence of EB species in the biofilm which actively boosted the electron transfer. The biomass of EB was also determined by volatile solid (VS) and colony-forming unit (cfu) methods in order to see the correlation between EB growths with the electricity generation and COD removal.

Kinetics and modeling for growth and COD removal were also carried out. Two unstructured kinetic models were proposed, namely Logistic and Modified Leudeking-Piret. The validity of the models was evaluated by determining the coefficient of determination (R^2), root mean square error (RMSE) and variance. Prediction on COD removal yield in case of fluctuation of the kinetic model parameters was done using Monte Carlo algorithm where the variability of these parameters are represented by normal distribution of random numbers. Meanwhile, the impact of each kinetic model parameters on the COD removal was also evaluated by sensitivity analysis. Then, six ML-MFCs were setup for a series circuit and the electricity generation and COD removal were observed. The real application of power harvested from the study was applied to several devices to determine the new renewable potential energy.

1.5 Novelty of study

The novelty resides in an unified approach that deals with the combination of substrate and inoculum of dewatered sludge inside the ML-MFC. The ML-MFC was simplified of a MFC as it was single chamber, membrane-less and mediator-less. Furthermore, the design experiment using the RSM, the evaluation of the two kinetic models plus with the uncertainty analysis on the COD removal inside the ML-MFC really become among the significant findings of the research.

1.6 Thesis Organization

There are five chapters in this thesis and each chapter described the sequences of the research that were carried out in this study.

Chapter 1 introduces the lack of access to clean electricity in developing nations and the existing technologies used to produce power. This chapter also highlighted the problem statement, research objectives, scope of research and thesis organization.

Chapter 2 covers an overview of different types of power generation based on non-renewable and renewable energy. Besides that, this chapter also reviews on related operational factors that may affect the performance theories, method and analyses. The theories for the mathematical model and simulation approach is also described in detail.

Chapter 3 highlight on the material and methods, describing the procedure for the growth of bacteria, COD removal and generation of electricity in ML- MFC. This chapter also covered the kinetic modeling studies and analytical procedures.

Chapter 4 presents the results and discussions on the experimental and modelling works that have been conducted. The kinetic model for biomass growth of electrogenic bacteria, COD removal, Monte Carlo simulation and sensitivity analysis in the ML-MFC were validated.

Chapter 5 covers the overall conclusions that are based on the major findings obtained in the results and discussion. Recommendations for future research are also given in the chapter.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

A developing country like Malaysia has high demand of energy due to rapid population growth and economic expansion (Rahim and Liwan, 2012) and it continuously increased every year. In order to achieve such demands in Malaysia, the effective and sustainable energy policy is very much important (Oh et al., 2010). Energy policy is a high-profile issue recently, with the focus on energy security, sources of fossil fuels, and alternative clean energy (Baicha et al., 2016). The main source of energy used most around the world at this time is derived from fossil fuels, but there are many issues associated with their use (Sen and Ganguly, 2017). Fossil fuels are not a sustainable source of energy that will be completely exhausted (Gude, 2016). Energy-saving technologies need to be developed to conserve oil reserves and at the same time more sustainable technologies need to be created in the next decade, and perhaps centuries (Ahmed et al., 2014; Branker et al., 2011). A sustainable energy specialization involving carbon technology and renewable energy should be developed. Reliance on renewable energy is growing; technologies such as solar, wind and biomass energy play an increasingly important role in meeting our energy future (Tommasi and Lombardelli, 2017).

Microbial fuel cell (MFC) converts chemical energy to electrical energy by certain microorganisms. Regarding to this, MFC are bio-electrical devices that harness the natural metabolism of electrogenic bacteria (EB) to produce electrical energy (Ren, 2014). Only EB are capable of transferring metabolically-generated electrons across their cell membranes to an external electrode (Li, 2014). MFC recently become more attractive because the substrate used in such systems could be almost any biodegradable organic matter, including domestic (Ren et al., 2014) and industrial wastewater (Gude, 2016). By using anaerobic digestion technique in MFC, there are great potentials for both renewable energy generation and waste remediation, such as it's clean energy and reduces the offset cost in term of electricity because no aeration and recirculation processes are needed. Furthermore anaerobic processes reduce the cost for sludge treatment due to anaerobic digestion which reduces the formation of sludge compared to aerobic process.

Commonly, the formation of sludge at the wastewater treatment plant was occurred as the bacteria during the aerobic process uses most of the energy in the organic matters. Unlike the MFC system, the EB only used part of the energy for their growth and the balance energy is directly converted to electricity (Figure 2.1) (Pham et al., 2006). Table 2.1 and 2.2 summarize different major consideration options for sludge handling and restrictions on sludge management based on the principles of environmental concern, respectively. While Table 2.3 lists the technical status for several methods for recovering energy from the sludge produced including the sludge incineration and gasification, pyrolysis of sludge, fermentation or anaerobic digestion (Oh et al., 2010).

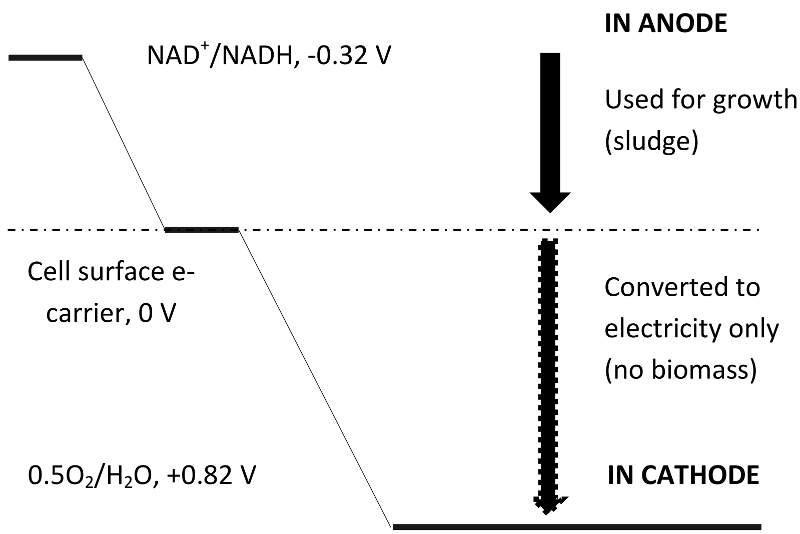

Figure 2.1 Energy conversions in a MFC (Rabaey and Verstraete, 2005)

Table 2.1 Illustration of different major consideration option for sludge handling (Fytili and Zabaniotou, 2008)

Option	Purpose	Application
No use	Stop use of an unwanted substance due to detrimental and irreversible effects to the environment	Effective control of industrial discharge, use of environmentally friendly consumer products to facilitate sludge use in agriculture and use of sludge product
Reuse	Decrease of the amount reaching the environment and extraction of mineral resources by reusing the compound	Internal reuse of materials (such as reuse of precipitation chemicals) and external reuse (such as the reuse of phosphorus as fertilizer)
Convert	Conversion of a substance from an obnoxious form into a form acceptable for further transport by air or water or into a solid form	Conversion of organics to methane gas (for further use as energy source), solubilisation of sludge components for product recovery, conversion of sludge into compost
Contain	To contain the residues with a low leaching ability as possible	Separate containment of toxic substances in the sludge, inclusion or stabilization of ashes from sludge incineration
Disperse	Dispersion into environment without negative impact	Effective dispersion of sludge in agricultural use, effective dispersion of untreated flue gases in sludge incineration

Table 2.2 Restriction on sludge management based on the principles of environmental friendly (Fytili and Zabaniotou, 2008)

Sludge handling method	Restriction based/due to/related
Agricultural use (similar restriction for horticulture and forest)	• Sludge components (metals, toxic organics) • Nutrients and metal supply to land • Acceptance from food industry and public • Technical restrictions (handling of the sludge)
Land deposition	• Maximum organic contents in the sludge • Costs based on fees • Scarcity of land • Permits of new land fill areas • Recycling requirement
Land building and reclamation	• Permits of building an incineration plant • Costs (including costs for treatment of flue gases and ashes)
Product recovery from sludge	• Acceptance from user of the sludge products (market considerations) • Needs of resources for product recovery (chemicals, energy and costs)

Table 2.3 Typical capacity and efficiency of clean energy technology to recover from wastewater (Oh et al., 2010).

Conversion option	**Technology**	**Types**	**Typical capacity**	**Net efficiency**	**Status**
Thermal process	Sludge combustion	Heat	Domestic boiler (1 - 5 MW)	Modern furnace 70-90 % domestic fireplace	The conventional furnace use still widely in Europe.
		Combine heat and power (CHP)	0.1 – 1MW	69 -90 %	Scandinavia, Australia are widely applied.
		Stand alone	1 - 10 MW 20 - 100 MW	80-100% 20 - 40% (electricity)	Conventional technology in Scandivia wide.
		Co-combustion	5 – 20 MW (electricity)	30 - 40	Commonly used by EU countries. Recent year, mostly considered as air pollution.
Biological process	Sludge gasification	Heat	5 - 20 MW(electricity)	10 - 15 heat content	Limitation: relatively high operating course, energy product
		Bio-oil	Several 100 kW	Several MW electricity	No commercialize Australis, Germany and France.
	Sludge pyrolysis	Landfill gas	100 kW (electricity)	Depend on gas engine efficient	Widely applied at EU.
		Microbial electrolysis cell	110 W/m^3 depend on H_2 cell fuel cell efficiency	82 % (UHV basis)	Produce hydrogen through electrolysis in biological reactor but the technology is still not possible to apply
	Anaerobic Digestion (Bioelectricity)	**Microbial fuel cell**	55 -1600 W/m^3	20-80 % columbic efficiency as the basis	**Promising technology where generating electricity while treating the wastewater**

2.2 Microbial Fuel Cell

Figure 2.2 illustrates the basic configuration and mechanism of the MFC. The first step occurrs in the MFC is the EB in the anodic chamber (anoxic condition) begin to oxidize the added substrate and release electrons (e^-) towards the anode, as well as the protons (H^+). Carbon dioxide (CO_2) is produced as a product of oxidation. Electrons produced are transported from the anode to the cathode through the external circuit to generate electricity. After passing the proton exchange membrane (PEM), the protons enter the cathode where oxygen (O_2) reduction occurs and water (H_2O) is formed (Saba et al., 2017; Saratale 2017). Reaction on anode and cathode electrodes in a typical MFC using acetate (CH_3COO^-) as substrate models is presented below (Lovley, 2008):

Anodic reaction: Acetate oxidation

$$CH_3COO^- + 2H_2O \longrightarrow 2CO_2 + 7H^+ + 8e^- \quad (2.1)$$

Cathodic reaction: Oxygen reduction

$$O_2 + 4e^- + 4H^+ \longrightarrow 2H_2O \quad (2.2)$$

MFC acts as a galvanic cell in which the anodic potential (E_{an}) is lower than the potential of the cathode (E_{cat}). Cell reactions occur spontaneously and as a result the electrical current is generated.

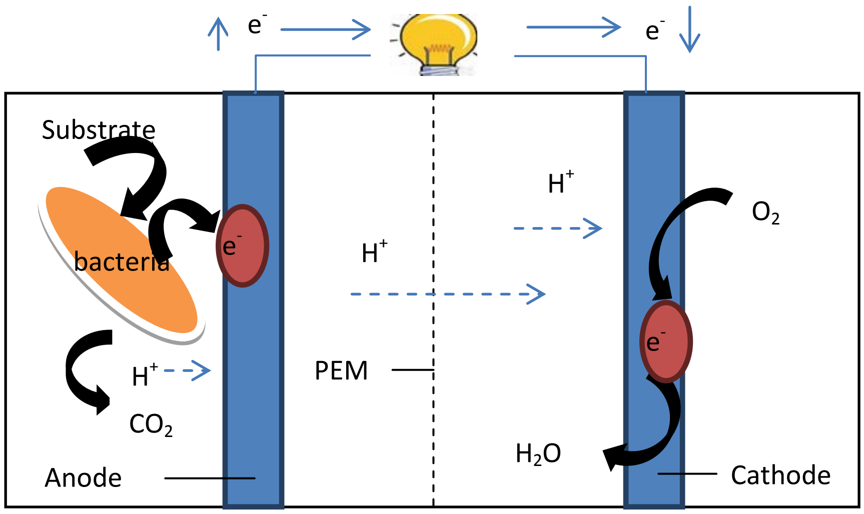

Figure 2.2 Basic configuration and mechanism of the MFC. (PEM: Proton exchange membrane)

2.2.1 Biological Concept in MFC

EB obtain carbon sources from a variety of organic compounds such as lipids, proteins and carbohydrates (Thomas et al., 2013). Lipids, carbohydrates and protein went through different reactions to convert them via glycolysis and related processes in the acetyl unit of acetyl-CoA and this is illustrated in Figure 2.3. Acetyl-CoA molecule will pass through the citric acid cycle, where oxidation reactions occur to decrease NAD^+ and FAD to form electron carriers, NADH and $FADH_2$. The citric acid cycle took place in the cytoplasm, and then the electron carrier, NADH and $FADH_2$, transfer the electron to the membrane layer (Wang et al., 2017). In fact, before it is sent to the terminal electron acceptor (oxygen), the electron need to go through different membrane proteins as clearly described in Figure 2.4. Some of them pump protons as reflect once they are reduced. There were energy of proton gradient and it was mediated through the ATP synthase transmembrane protein. The energy being used by the cell to produce ATP (chemical energy for living organism) resulting from the phosphorylation process of ADP (Figure 2.4). The reduction of terminal electron recipient inorganic generated the ATP and this process is called respiration (Zhang et al., 2017). In MFC system, EB can replace the electrode as terminal electron recipient anodic compartment in the reactor.

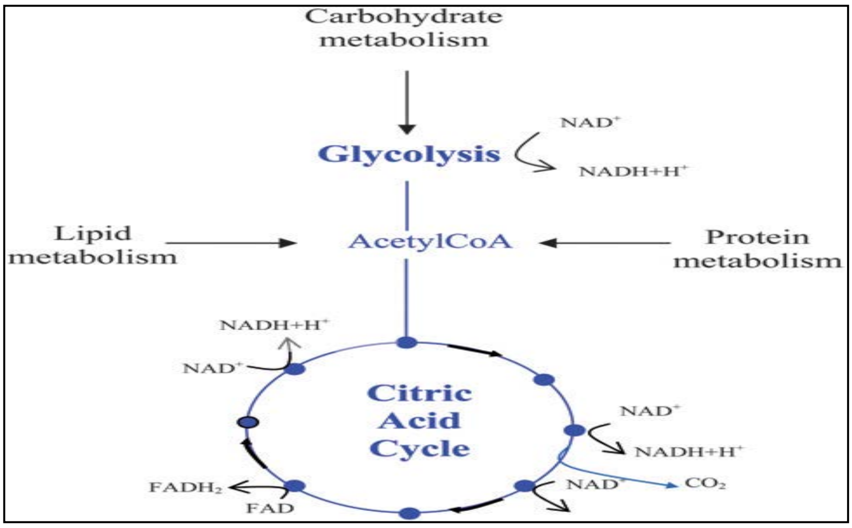

Figure 2.3 Glycolysis and citric Acid Cycle processes where electron carrier (NADH and $FADH_2$) forms (Schaetzle et al., 2008).

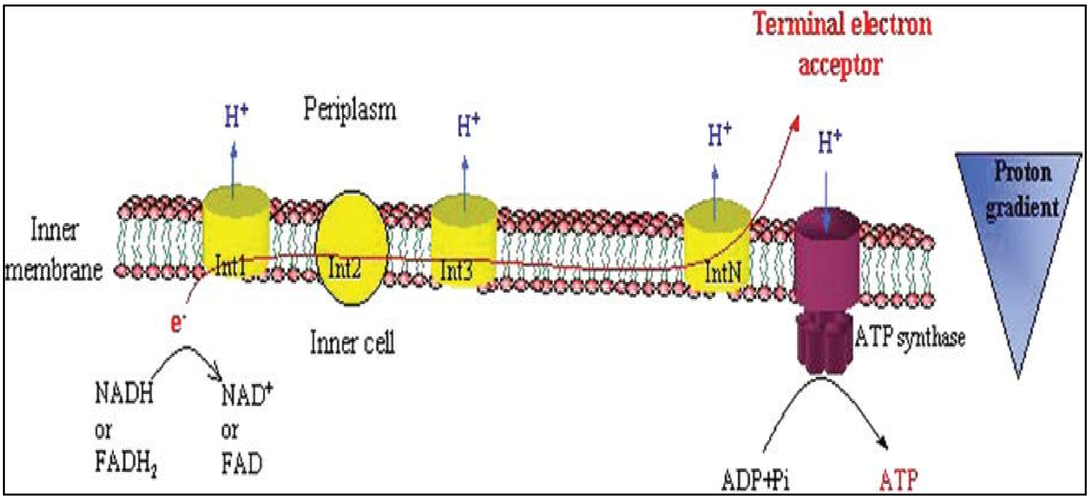

Figure 2.4 Schematic representation of bacterial membrane respiration. The number of components of the electron transport chain varies with species (Schaetzle, 2008).

2.2.2 Chemical concept in MFC

The difference in redox potential between two distinct electrodes is the key to determine the MFC voltage (Zhi et al., 2014). Microbial grew up forming the colonies and started to respire on the surface of the anode, at the same time highly reduced biomolecule began to accumulate and surrounded the anode. The build-up of the reduced biomolecule resulted a decrement of electrical potential (commonly around 0.1 V until -0.4 V vs standard hydrogen electrode (SHE)). While the cathode is placed at enriched oxygen environment and this situation created a higher electrical potential (commonly around 0.4 V to 0.8 V vs SHE) (Cheng and Logan, 2011). Thus, the working voltage could be calculated by subtracting the anode potential from the cathode potential. Maximum voltage that can be achieved between two electrodes are approximately 1.2 V (theoretically) due to the minimum potential of reduced molecules is – 0.4 V vs SHE and the redox potential of oxygen is 0.8 V. Figure 2.5 shows how the voltage is being determined by depending on the location of exit chain of respiratory enzyme (Zhi et al., 2014). Table 2.4 represents MFC electrode reactions and corresponding redox potential.

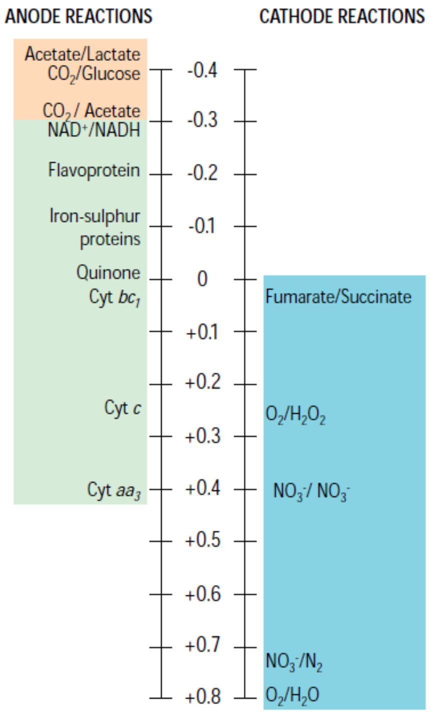

Figure 2.5 The respiratory chain and standard potential (Watanabe, 2008)

Table 2.4 MFC electrode reactions and corresponding redox potential (Watanabe, 2008)

Oxidation/reduction pairs	**E^o(mV)**
H^+/H_2	-420
NAD^+/NADH	-320
So/HS-	-270
SO_4^{2-}/H_2S	-220
Pyruvate^{2-}/Lactate^{2-}	-185
2,6-AQDS/2,6-AHQDS	-184
FAD/$FADH_2$	-180
Menaquinone ox/red	-75
Pyocyanin	-34
Humic substances ox/red	-200 to +300
Methylene blue ox/red	+11
Fumarate^{2-}/Succinate	+31
Thionine ox/red	+64
Cythochrome b (Fe^{3+})/ Cythocrome b (Fe^{2+})	+75
Fe(III) EDTA/Fe(II) EDTA	+96
Ubiquinone ox/red	+113
Cythrochrome c (Fe^{3+})/Cythochrome c(Fe^{2+})	+254
O_2/H_2O_2	+275
Fe(III) citrate/Fe(II) citrate	+372
Fe(III) NTA/Fe(II) NTA	+385
NO_3^-/NO_2	+421
Fe(CN)6$3^-$/Fe(CN)6	+430
NO_2^-/NH_4^+	+440
O_2/H_2O	+820

2.2.3 Characteristic for EB in the MFC

EB is a main player in the MFC as it has ability of transferring the electron outside their cell. Thus, investigation in the field of bio-electricity production had focused on the identification and isolation of bacteria that have the ability to transfer electrons to the electrode (Zou et al., 2017). Electrons from the EB accepted by the anode acted as a terminal electron acceptor in the anoxic environment (absence of oxygen) (He et al., 2015). EB shunts away electrons produced from the oxidation of organic compounds. *Shewanella* group species is the first organism reported to transfer electrons to the electrode surface (Zheng et al., 2017). There are three methods to transfer the electron to the anode as shown in Figure 2.6 which are direct transfer from the cell walls of microbes to the anode surface, using biomolecules secondary to shuttle electrons to the anode and lastly the transfer of electrons through a conductive appendages, called "nanowires", planted by microbes (He et al., 2015; Wang et al., 2017; Zhi et al., 2014).

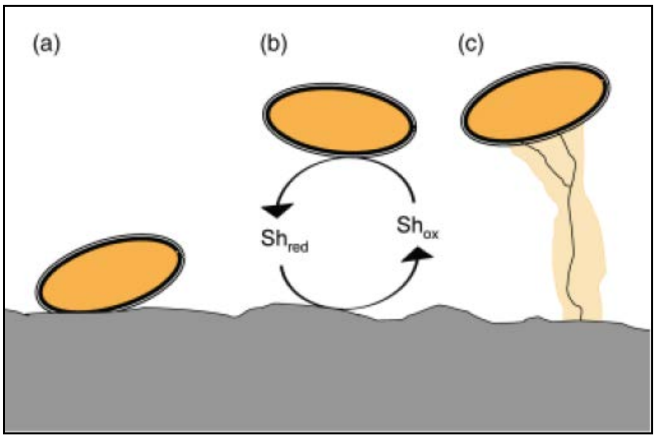

Figure 2.6 Electron transport in microbial fuel cells (a) direct electron transfer, (b) an electron shuttling and (c) solid conductive appendages, called "nanowires" (Rabaey and Verstraete, 2005)

2.2.3 a) Axenic bacterial culture

MFC can be operated using axenic bacterial culture or in mixed culture. Axenic bacterial describes that the MFC operates with pure culture. The single species of EB in MFCs, typically, they could easily be found in sediments where they utilized Fe (III) and Mn (IV) as the insoluble electron acceptors. For *Shewanella putrefaciens*, it has a special feature where there is specific cytochrome outside the cell membrane that allows active electrochemical in case it is grown under anaerobic conditions. Family of *Geobacteraceae* bacteria also have the same capability to survive in an aerobic environment and it had been investigated that they formed a layer of biofilm at anode electrode in the MFC to transfer electrons from the acetate with high efficiency (Roy, 2017).

In the anoxic sediment, there is *Rhodoferax* species which had high efficiency in transferring electrons to the graphite anode using glucose as the carbon source (Chaudhuri and Lovley, 2003). This was the first EB strain reported capable to complete mineralize glucose to CO_2 and at the same time generates electricity at 90% efficiency (Kim et al., 2005; Lovley, 2008). From the perspective of performance, EB such Shewanella *putrefaciens, Geobacter sulfurreducens* and *Rhodoferax ferrireducens* were able to produce current densities in the range of 0.2 - 0.6 mA and 1- 17 mW power density of surface / m^2 in conventional (woven) graphite electrodes (Chaudhuri and Lovley, 2003). These EB generally showed high efficiency of electron transfer but the disadvantages of axenic EB culture is that they had slow growth rate and high substrate specificity (especially for acetate or lactate) (Rabaey and Verstraete, 2005). Besides that, comparing to the mixed EB culture, they are relatively low efficiency in term of energy transfer. Furthermore, the high potential to be contaminated with unwanted bacteria inside the MFC is also one of the major obstacles when dealing with a pure EB culture (Roy, et al., 2017).

2.2.3b) Mixed EB culture

MFC using mixed bacterial cultures have a number of significant advantages over MFC using a pure culture. They have much higher resistance to interference process, higher substrate intake rates, low substrate specificity and higher power generation (Santoro et al., 2017; Wang et al., 2017). Electrochemically active mixed EB culture mostly came from the sediment of sea and lake or from the activated sludge at wastewater treatment plant. Example of EB species of electrochemically active species are *Geobacteraceae*, *Desulfuromonas*, *Alcaligenesfaecalis*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Proteobacteria*, *Clostridia*, *Bacteroides*and *Aeromonas* species. It is also concluded that the nitrogen fixing EB (e.g., *Azoarcus* and *Azospirillum*) (Kim et al., 2005) and the sludge that went through methanogenesis process, after continuously harvested for its anodic population over a 5-month period using glucose as carbon source were amongst the electrochemically active EB populations (Rabaey and Verstraete, 2005). There are also facultative anaerobic EB such as *Alcaligenes*, *Enterococcus* and *Pseudomonas* species. Table 2.5 below shows the power output delivered by MFC using different types of EB.