ELECTRICITY GENERATION BY *Pseudomonas aeruginosa* ZH1 IN MICROBIAL FUEL CELL USING PALM OIL MILL EFFLUENT

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For My Mother, My Father and My Country

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ABSTRACT

Microbial fuel cell (MFC) is a bioelectrochemical system that is recognised as a promising source of renewable energy. Electro-active bacteria are used to generate electricity in MFC, either as pure or mixed culture. Recent studies have shown that MFC is capable of utilising various types of wastewaters as the substrate for electricity generation. In view of this, the investigation on the feasibility of pure bacterial culture for electricity generation in a double-chambered MFC using final discharge palm oil mill effluent (POME) was carried out. The physical enhancement method was used to isolate electro-active bacteria from POME sludge grown on the anode of MFC. The isolate was identified and designated as *Pseudomonas aeruginosa* ZH1 using the 16S rRNA gene sequence analysis. The maximum power density and current density generated in MFC using *P. aeruginosa* ZH1 were 451.26 ± 22.97 mW/m^2 and 654.90 ± 17.12 mA/m^2, respectively. The high electricity generation was contributed from the self-produced pyocyanin which acted as the electron mediator. Analysis on the biochemical and physical factors affecting the MFC performance showed that *P. aeruginosa* ZH1 could not achieve high electricity generation and efficient treatment of POME simultaneously in MFC. Significant electricity generation was achieved at initial anode pH 9, external resistance of 500 Ω, 10% (v/v) inoculum size, under facultative anaerobic condition, undiluted POME as the substrate, using graphite felt as the electrodes with a surface area of 24.84 cm^2 and the addition of pyruvate and yeast extract in the anode. Furthermore, the time-course characterisation method was conducted to analyse the performance of MFC at 4, 24, 72 and 120 hours, respectively under batch mode operation. The maximum power generation and polarisation curve indicated that the optimum MFC performance was achieved at 72 hours. This was in correlation with the optimum biofilm development at 72 hours as observed from the bacterial concentration, microscopic imaging and Fourier Transform Infra-Red spectroscopy (FTIR) analysis. The long term MFC performance was investigated under sequential batch mode for 25 days at five days/cycle. The addition of pyruvate and yeast extract increased the electricity generation in which the maximum power density and current density were achieved during the second cycle at 33.51 ± 30.35 mW/m^2 and 153.40 ± 68.90 mA/m^2, respectively. Microscopic and elemental analysis revealed that the developed biofilm consists of web-like structures which could represent bacterial nanowires for extracellular electron transfer. In conclusion, this study demonstrated that *P. aeruginosa* ZH1 is a suitable electro-active bacteria for the generation of electricity in MFC using final discharge POME.

ABSTRAK

Sel fuel mikrob (SFM) merupakan sistem bioelektrokimia yang dikenalpasti sebagai sumber tenaga diperbaharui yang berpotensi. Bakteria elektro-aktif digunakan untuk menjana elektrik dalam SFM, sama ada sebagai kultur tulen atau kultur campuran. Kajian terkini menunjukkan bahawa SFM mampu menggunakan pelbagai jenis air sisa sebagai substrat untuk penjanaan elektrik. Berdasarkan pemerhatian ini, penyelidikan kebolehlaksanaan kultur tulen bakteria untuk penjanaan elektrik dalam dwi-kebuk SFM menggunakan luahan akhir efluen kilang minyak sawit (EKMS) telah dijalankan. Kaedah peningkatan fizikal digunapakai untuk mengasingkan bakteria elektro-aktif dari enapcemar EKMS yang tumbuh pada anod SFM. Bakteria terpencil telah dikenalpasti dan dinamakan sebagai *Pseudomonas aeruginosa* ZH1 melalui analisa susunan gen 16S rRNA. Ketumpatan kuasa dan ketumpatan arus maksimum yang dijana dalam SFM menggunakan *P. aeruginosa* ZH1 masing-masing adalah 451.26 ± 22.97 mW/m^2 dan 654.90 ± 17.12 mA/m^2. Penjanaan elektrik yang tinggi ini disumbangkan oleh pyocyanin hasilan sendiri yang berfungsi sebagai pengantara elektron. Analisa terhadap faktor biokimia dan fizikal yang mempengaruhi prestasi SFM menunjukkan *P. aeruginosa* ZH1 tidak dapat mencapai penjanaan elektrik yang tinggi dan rawatan EKMS yang efisien secara serentak. Penjanaan elektrik yang ketara dicapai pada permulaan anod pH 9, rintangan luar pada 500 Ω, 10% (v/v) saiz inokulum, di bawah keadaan fakultatif anaerob, EKMS tanpa pencairan sebagai substrat, menggunakan felt grafit sebagai elektrod dengan luas permukaan 24.84 cm^2 dan penambahan piruvat dan ekstrak yis dalam anod. Selanjutnya, kaedah pencirian haluan masa dijalankan untuk menganalisa prestasi SFM pada 4, 24, 72 dan 120 jam di bawah operasi kultur kelompok. Penjanaan tenaga maksimum dan lengkung polarisasi menunjukkan prestasi optimum SFM dicapai pada jam ke 72. Analisa ini sejajar dengan pembentukan biofilem pada jam ke 72 sebagaimana yang dilihat daripada kepekatan bakteria, imej mikroskopik dan analisa jelmaan Fourier infra-merah (FTIR). Prestasi jangka panjang SFM dijalankan di bawah mod jujukan kelompok selama 25 hari dengan lima hari/kitaran. Penambahan piruvat dan ekstrak yis meningkatkan penjanaan elektrik di mana ketumpatan kuasa dan ketumpatan arus maksimum dicapai semasa kitaran kedua masing-masing pada 33.51 ± 30.35 mW/m^2 dan 153.40 ± 68.90 mA/m^2. Analisa mikroskopik dan analisa unsur menunjukkan biofilem yang terbentuk terdiri daripada struktur yang menyerupai sarang labah-labah yang mungkin mewakili wayar-nano bakteria untuk pemindahan elektron ekstraselular. Kesimpulannya, kajian ini menunjukkan bahawa *P. aeruginosa* ZH1 adalah bakteria elektro-aktif yang sesuai untuk penjanaan elektrik dalam SFM menggunakan luahan akhir EKMS.

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LIST OF ABBREVIATIONS

ADMI	-	American Dye Manufacturer Institute
AFM	-	Atomic force microscope
ATP	-	Adenosine triphosphate
BES	-	Bioelectrochemical system
BET	-	Brunauer-Emmet-Teller
BLAST	-	Basic Local Alignment Search Tool
BOD	-	Biochemical oxygen demand
bp	-	Base pair
BSA	-	Bovine serum albumin
COD	-	Chemical oxygen demand
DNA	-	Deoxyribonucleic acid
DNS	-	Dinitrosalicylic acid
DO	-	Dissolved oxygen
EET	-	Extracellular electron transfer
EFB	-	Empty fruit bunch
EPS	-	Extracellular polymeric substance
FESEM	-	Field emission scanning electron microscope
FFB	-	Fresh fruit bunch
FTIR	-	Fourier transform infrared
GDP	-	Gross domestic product
GHG	-	Greenhouse gas
HCl	-	Hydrochloric acid
HPLC	-	High performance liquid chromatography
IQR	-	Interquartile range
kb	-	Kilo base
MDC	-	Microbial desalination cell
MEC	-	Microbial electrolysis cell

MFC	-	Microbial fuel cell
NaOH	-	Sodium hydroxide
NADH	-	Nicotinamide adenine dinucleotide
NAD(P)H	-	Nicotinamide adenine dinucleotide phosphate
NREP	-	National Renewable Energy Policy
PEM	-	Proton exchange membrane
PMFC	-	Plant microbial fuel cell
POME	-	Palm oil mill effluent
rRNA	-	Ribosomal RNA
SEM	-	Scanning electron microscope
UV	-	Ultraviolet

LIST OF SYMBOLS

A	-	Ampere (Current)
$E_{1/2}$	-	Mid-point potential
E_{pa}	-	Reduction peak potential
E_{pc}	-	Oxidation peak potential
g	-	Gram
g/L	-	Gram per litre
Hz	-	Hertz
i_{pa}	-	Anodic peak current
i_{pc}	-	Cathodic peak current
kPa	-	Kilo pascal
ktoe	-	Kilo tonnes of oil equivalent
L	-	Litre
M	-	Molarity
m	-	Median
mA/m^2	-	Current density
max_p	-	Maximum point
mg/L	-	milligram per litre
min_p	-	Minimum point
mm	-	Millimetre
mW/m^2	-	Power density
nm	-	Nanometer
Q_1	-	Lower quartile
Q_3	-	Upper quartile
rpm	-	Rotation per minutes
S	-	Conductivity
scf	-	Standard cubic feet (of gas)
Tm	-	Melting temperature

V	-	Voltage
v/v	-	Volume over volume percentage
w/v	-	Weight over volume percentage
ΔE_o	-	Peak to peak separation
Ω	-	Ohm
%	-	Percentage
°C	-	Degree Celsius

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Palm oil is one of the most valuable and consumed vegetable oil in the world with a worldwide production of 65.5 million tonnes in 2016/2017 (FAO, 2018; Jiménez-Carvelo *et al.*, 2017; Statistica, 2018). The cheaper production cost of palm oil compared to other alternative vegetable oils (Basiron, 2008; Basiron and Simeh, 2005; Mobin Siddique *et al.*, 2010), the capability to yield high amount and good quality of vegetable oil (an average of 10 times higher yield compared to other oil crops such as soybean, rapeseed and sunflower) (MPIC, 2011; SDP, 2014) and its low requirement for cultivation area which occupies less than 5% of the total world's agricultural land (Khatun *et al.*, 2017; Lam and Lee, 2011) are among the factors that contributed to the successful development of the palm oil industry, subsequently meeting the huge global demand for palm oil.

The significant progress of the palm oil industry in particular has turned Malaysia into one of the three world's largest producers and exporter of palm oil and its products, together with Indonesia and Thailand (Silalertruksa *et al.*, 2017; van Duijn, 2013). From a total area of 1.48 million hectares of oil palm cultivated in 1985, the total acreage under oil palm in Malaysia has expanded to 5.74 million hectares by 2016 (MPOB, 2010 and 2016). As a result, the sum production of palm oil in Malaysia have increased constantly from 4.1 million tonnes of palm oil in 1985 to 10.6 million tonnes in 1999, 18.9 million tonnes in 2011 and 21.0 million tonnes in 2016 (Hosseini and Abdul Wahid, 2015; Indexmundi, 2016; Neoh *et al.*, 2013).

The extraction of crude palm oil involves the boiling of fresh fruit bunch (FFB), which requires a significantly huge amount of water during the process. It was estimated that each tonne of crude palm oil produced required 5 – 7.5 tonnes of water, with more than 50% of this water ends up as palm oil mill effluent (POME) (Wu *et al.*, 2009; Yacob *et al.*, 2006). POME is a mixed colloidal suspension consisting of 95 – 96% water, 0.6 – 0.7% oil and 4 – 5% total solids (Ahmad *et al.*, 2006a; Onyia *et al.*, 2001). The chemical oxygen demand (COD) and biological oxygen demand (BOD) level in POME exceeds well over the value set by the Department of Environment (DOE). In addition, POME has a brownish colour with a discharged temperature of between 80 – 90°C and an acidic property around pH 4.0 – 5.0 (Bala *et al.*, 2014a; Ma, 2000).

Despite the overwhelming development of Malaysian palm oil industry, the amount of POME produced also increases in proportion to the world demand of edible oil (Ahmed *et al.*, 2015; Bala *et al.*, 2015). The excessive level of COD and BOD in POME could severely harm the aquatic life forms in rivers and water stream (Borja *et al.*, 1996; Loh *et al.*, 2013). In addition, the intense colour of POME would prevent sunlight from penetrating into the water, thus endangering photosynthetic organisms in the water (Azreen *et al.*, 2017; Okwute and R. Isu, 2007). This leads to a growing demand by the palm oil mill industry for a sustainable treatment of POME in order to minimise the pollution. With over 430 palm oil mills across the country producing POME in large amount every day (Cheng *et al.*, 2010; Gobi and Vadivelu, 2013), the discharge of POME into the rivers present a potential threat to the ecosystem and environment.

Malaysia is also well known as one of the producers of petroleum oil and natural gas. In 2016, Malaysia was ranked 25-26th in the world in terms of crude oil production and 23-25th in terms of natural gas production (CIA, 2018; EIA, 2016). Besides oil and gas, the main energy sources in Malaysia come from coal and a small portion of renewable energy sources; hydroelectricity, biomass and solar energy (Oh *et al.*, 2018; Shaikh *et al.*, 2017). The country depends heavily on the availability of cheap energy supply for continuous growth such that the GDP growth showed a relationship almost exactly with the energy consumption of the country (Hamid *et* *al.*, 2008; Shafie *et al.*, 2011). Therefore, energy-related policy measures have been a top priority to ensure the energy security and sustainability in the country (Chien Bong *et al.*, 2017; Yatim *et al.*, 2016).

On a global perspective, the excessive consumption of fossil fuels have resulted in a serious global climate change as well as growing concern over the possible depletion of fossil fuel supply (Shafiee and Topal, 2009; Singh and Singh, 2012). Even with the recent drop of global oil price, fossil fuels still play a crucial role in the world energy market such that prices are expected to recover over the course of the projection period, reaching between USD 141 – 226 per barrel by 2040 (EIA, 2017a; IEO, 2016). In line with the 1972 Montreal and 1997 Kyoto protocols, the Malaysian government had acknowledged green technology application as a long term and practical solution towards climate change and energy security (KeTTHA, 2009; Mekhilef *et al.*, 2014; Shi *et al.*, 2013). The country is actively pursuing in research and development to utilise various sources of renewable energy including biomass wastes, solid wastes and landfill gas, hydropower, solar power, and wind energy to support its energy demand and consumption (Kardooni *et al.*, 2016; Shekarchian *et al.*, 2011).

Microbial fuel cell (MFC) is one of the rapidly developing renewable energy technologies which have received increasing attention for its promising and potential application as an alternative source of renewable energy. MFC or formerly known as biological fuel cell is a bioelectrochemical device that utilises unique electro-active microorganisms to convert chemical energy directly into electrical energy (Du *et al.*, 2007; Merino Jimenez *et al.*, 2017; Sun *et al.*, 2016). The basic principle of MFC is the extracellular transfer of electrons from the electro-active microorganisms to the anode and through the external circuit to reach the cathode (Ieropoulos *et al.*, 2005; Rahimnejad *et al.*, 2015; Schroder, 2007). Current is generated during the movement of electrons from the anode to cathode due to the difference in redox potential that exists between the dissimilar liquid solutions.

MFC offers a number of advantages over other renewable energy. Mainly, its direct conversion of chemical energy within the substrate to electricity allows a higher energy conversion efficiency without any combustion process (Cheng *et al.*, 2010; Lee *et al.*, 2008; Rahimnejad *et al.*, 2012). In terms of cost of operation, MFC does not require any energy input to drive the system for its operation (Liu and Logan, 2004; Rabaey and Verstraete, 2005). The system is also suitable at ambient temperature, thus avoiding huge energy consumption to stabilise the temperature of the system (Gude, 2016; Schroder, 2007). In addition, the use of microorganisms in MFC for electricity generation eliminates the high cost of constructing the MFC compared to the cost of using metal catalyst or extracted enzymes (Rahimnejad *et al.*, 2012; Santoro *et al.*, 2017; Scott and Murano, 2007). Furthermore, low sludge yield in MFC compared to other wastewater treatment systems such as anaerobic digestion (He *et al.*, 2017; Rozendal *et al.*, 2008a) or activated sludge (Jang *et al.*, 2004; Ren *et al.*, 2012) offers a much lower cost to manage and dispose sludge.

Over the years, research on sustainable energy has been focusing on wastes material as a source of renewable energy that is cheap, abundant and attainable. The ability of the electro-active microbial communities to harvest and extract clean energy from waste organic sources rather than filtering the contaminants have allowed MFC to utilise many different types of wastewater, ranging from domestic wastes (Mohan *et al.*, 2009; Xing *et al.*, 2008), industrial wastes (Huang and Logan, 2008a; Patil *et al.*, 2009), agricultural wastes (Guo *et al.*, 2013; Kaewkannetra *et al.*, 2011; Lim *et al.*, 2012) and animal wastes (Jadhav *et al.*, 2016; Kim *et al.*, 2008; Min *et al.*, 2005b). Given that POME is not only rich in organic matters (Ji Chin *et al.*, 2013; Loh *et al.*, 2013) but also abundantly available at a very low price (Aliyu and Md. Zahangir, 2012), POME has been recognised as a promising source of waste material for electricity generation in MFC (Baranitharan *et al.*, 2015a; Jong *et al.*, 2011; Tee *et al.*, 2017). Therefore, MFC is seen as an ideal and potential green technology that can generate clean and renewable electricity from the treatment of wastewater (Badwal *et al.*, 2014; Sun *et al.*, 2016).

Although the energy production in MFC is still low compared to other fuel cell technologies (Knight *et al.*, 2013; Pham *et al.*, 2009), the development of MFC have drawn huge attention from the scientific community in the past decade. The rising interests have become a major driving force towards increasing the understanding and achieving further breakthroughs with the goal to transform MFC into a practical source of sustainable energy-harvesting technology. In short, MFC technology appears to be one of the most exciting areas of future research which provide not only a new platform to understand less explored microbial physiological and molecular mechanisms but also present a new and promising biological approach for solving the environmental pollution problem and energy crisis with a unified approach (Kumar *et al.*, 2016; Venkata Mohan *et al.*, 2014).

1.2 Problem Statement

To date, most MFC studies that utilised POME as the substrate have focused on mixed natural microflora from POME sludge as the inoculum. Despite the practical approach of using POME in MFC, the complex interactions between the microbial communities were not well understood, hence resulting in difficulty to interpret and analyse the overall production of electricity and treatment of POME. In view of this, previous studies have proposed different techniques to isolate and select potential electro-active bacteria from the environment. Since POME sludge consists of various microorganisms, thus, it is vital to investigate the isolation and selection of pure bacterial culture from POME sludge for the generation of electricity in MFC.

In addition, there are many factors that can affect the performance of MFC, including physical and biochemical parameters. The maximum potential energy generation in MFC could not be achieved without a thorough understanding on the parameters that influence MFC performance. Furthermore, the performance of MFC is normally studied once the MFC reached a steady state based on voltage or current profiles. Nevertheless, the results could not be related to the time-varying biocatalyst activity and changes in the performance trend under microbial meaningful timescales. More importantly, continuous electricity cannot be obtained from MFC operated in batch mode. Therefore, it is crucial to explore the prospect of continuous generation of electricity in MFC using alternative modes of operation so that a sustainable source of electricity production can be achieved in MFC for long-term practical application.

1.3 Objectives of the Study

The main objective of this study was to investigate the feasibility of using pure bacterial culture isolated from POME sludge for electricity generation using final discharged POME as the substrate in a double-chambered MFC. The objectives of the study were as follows:

1) To isolate and characterise potential electro-active bacteria from POME sludge
2) To identify the physical and biochemical factors that influence the electricity generation and treatment of POME in MFC
3) To analyse the performance of MFC using the time-course characterisation method in batch mode operation
4) To evaluate long term performance of MFC through sequential batch mode operation

1.4 Scope of the Study

It was suggested that POME has a huge potential to be utilised as a source of substrate for the generation of electricity in MFC. Therefore, this study was focused on the development of a double-chambered MFC utilising pure culture bacteria for the generation of electricity using final POME as the substrate. The physical enhancement method was performed to isolate and select potential electro-active pure bacterial culture from POME sludge in a double-chambered MFC. The bacteria was selected based on their ability to adapt in the fuel cell environment with final POME as the substrate as well as developing the biofilm on the anode. The selected electro-active bacteria was characterised and identified using 16S rRNA gene sequence analysis. The ability of the selected bacteria to produce electron mediator was also determined. The performance of the selected bacteria to generate electricity and treat POME in was then investigated. The MFC performance was also performed using mixed natural microflora from POME sludge as the inoculum.

A series of MFC operations were also conducted to identify the physical and biochemical parameters that affects the electricity generation and wastewater treatment in MFC. The parameters included initial anode pH, external resistance, type and size of electrode. Electricity generation and water quality analysis were analysed for each parameter. Furthermore, the time-course characterisation method was conducted to study the relationship between anode microbial characteristics and electrochemical parameters in MFC. The characteristics were analysed over a selected time interval through electricity generation, water quality and biofilm development on the anode. Finally, the continuous electricity generation under long term MFC operation was investigated. The principal operation of sequential batch reactor (SBR) which involves the fill-and-draw process was applied during a 25 day MFC operation using final POME as the substrate. Besides electricity generation and water quality analyses, the biofilm development on the anode was analysed. The assessment of the MFC system for long term operation concluded the study.

1.5 Significance of the Study

The current global energy demand and consumption have made the development of MFC as an alternative renewable energy highly significant. In fact, the use of POME would serve as a potential source of substrate for electricity generation in MFC offers an attractive solution towards the increasing discharge of POME from the palm oil industry. Therefore, the use of POME as a complex substrate for electricity generation in MFC showed promising results in this study. The isolation and characterisation of potential electro-active bacteria in this study have provided new understanding on the ability of specific bacteria to generate electricity. With the use of single culture bacteria in MFC, the factors affecting MFC and the characterisation of MFC performance were efficiently investigated. Most importantly, the long term MFC operation under sequential batch mode operation showed that the MFC can generate continuous electricity over a long period of time using POME as the substrate. It is hopeful that MFC technology would contribute a significant role to meet the energy demand as well as providing an alternative solution for a sustainable treatment of POME.

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