ZINC-AIR MICROBIAL FUEL CELL FROM FUNGAL DEGRADATION OF OIL PALM EMPTY FRUIT BUNCH

BY

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ABSTRACT

The main stumbling block for practical implementation of bioenergy harvesting is the low energy gain yields (output/cost). Therefore, the challenge is to reduce the complexity of the cell design, minimize its control features and at the same time increase the energy output. The present work describes a bioelectrochemical system that adopts simple design configurations, operates in uncontrolled ambient surrounding and sustains a constant current output 1mA for 44 days. The microbial fuel cell (MFC) comprises of white rot fungus of *Phanaerochaete chrysosporium* fed with oil palm empty fruit bunch (EFB) agrowaste as the substrate. Unlike most MFCs, the fungal inoculums were not cultured on the current collector but left to be freely suspended in unbuffered potato dextrose broth (PDB) electrolyte. This fungal strain degrades lignin by producing ligninolytic enzymes such as laccase. Laccase demonstrates specific affinity for oxygen as its electron acceptor. By simply pairing zinc and air electrode in a membraneless, single chamber 250 ml enclosure, electricity could be harvested as the fungal microbes degrade the lignin-rich agrowaste. The microbial zinc/air cell was capable to sustain a 1 mA discharge current for 44 days continuously i.e. a 1056 mAh discharge capacity. The role of metabolic activities of *P. chrysosporium* on EFB towards the MFC performance was supported by linear sweep voltammetry measurement and scanning electron microscopy observations on lignin transformation during degradation. Scaling-up the bioelectrochemical system pose yet another challenge because biological processes of living microbes cannot be simply enhanced by incorporating more nutrients or substrate. This work investigated several aspects to increase the power density of an MFC. The factors studied were cathode surface area, volumetric capacity, cell stacking (series and parallel) and also a novel cell configuration. By increasing the cathode surface area substantially and pairing the cells in parallel, a 20-litre MFC prototype could deliver a 24 Ah discharge capacity, rated at 20 mA. The MFC however, is not economically viable. Using a novel cell configuration, the dependency on the air cathode was reduced and yet the MFC possessed much better discharge performance. A 5-litre prototype rated at 5 mA, demonstrated a discharge capacity of 2.2 Ah with an average operating voltage of 0.8 V. In conclusion, novel cell configuration is the most suitable design for scale up as it require less air cathode, smaller size and less volumetric capacity.

خلاصة البحث

يُعد العائق الرئيسي للتنفيذ العملي لحصاد الطاقة الحيوية هو انخفاض إنتاجية الطاقة (الناتج / التكلفة). لذلك، فإن التحدي يتمثل في تقليل مستوى تعقيد تصميم الخلية وميزات التحكم الخاصة بها وفي نفس الوقت زيادة إنتاج الطاقة. تمثل هذه الدراسة نظامًا بيولوجيًا كيميائيًا يتبنى تكوينات تصميم بسيطة، ويعمل في محيط غير متحكم فيه ويحافظ على إنتاج تيار مستمر يبلغ 1 مللي أمبير لمدة 44 يومًا. تتكون خلية الوقود الميكروبية (**MFC**) من فطر العفن الأبيض من ***Phanaerochaete chrysosporium*** والذي يتم تغذيته بالنفايات الزراعية لزيت نخيل حزمة الفكهة الفارغة (**EFB**) كركيزة. على عكس معظم خلايا الوقود الميكروبية، لم يتم زراعة اللقاحات الفطرية على المجمّع الحالي ولكن تم تركها بحرية في أغار البطاطس بالدكستروز (**PDB**) غير المصقول. تعمل هذه السلالة الفطرية على تحليل اللجنين عن طريق إنتاج إنزيمات تحلل اللجنين مثل اللاكيز، والذي يحمل ألفة معينة للأكسجين كمستقبل للإلكترون. عند جمع إلكترود الزنك والهواء في حاوية بدون غشاء تبلغ سعتها 250 مل، يمكن حصاد الكهرباء والسبب في ذلك هو أن الميكروبات الفطرية تعمل على تحليل النفايات الزراعية الغنية باللجنين. أظهرت خلية الزنك/ الهواء الميكروبية قدرتها على الحفاظ على تيار تفريغ يبلغ 1 مللي أمبير لمدة 44 يومًا بشكل مستمر، أي قدرة تفريغ تصل إلى 1056 مللي أمبير في الساعة. تم دعم دور الأنشطة الأيضية لـ ***P. chrysosporium*** على حزمة الفاكهة الفارغة فيما يتعلق بأدائها من خلال قياس الفولتميتر الخطي وقراءة ملاحظات المجهر الإلكتروني على تحول اللجنين أثناء التحليل. يشكل توسيع نطاق النظام الكيميائي الحيوي تحديًا آخر لأن العمليات البيولوجية للميكروبات الحية لا يمكن تعزيزها ببساطة عن طريق دمج المزيد من العناصر الغذائية أو الركائز. تحققت هذه الدراسة من عدة جوانب لزيادة كثافة الطاقة في خلية الوقود الميكروبية. وشملت العوامل المدروسة مساحة سطح الكاثود، والسعة الحجمية، وتكدس الخلايا (بشكل متسلسل ومتوازي) وكذلك التكوين الجديد للخلايا. من خلال الزيادة الكبيرة لمساحة سطح الكاثود وإقران الخلايا بشكل متوازي،

يمكن توفير نموذج أولي لخلية الوقود الميكروبية يبلغ سعته 20 لترًا، وذو سعة تفريغ تبلغ 24 ساعة، وتم تقييمها على أنها 20 مللي أمبير. ومع ذلك، فإن خلية الوقود الميكروبية ليست مناسبة اقتصاديًا. فعند استخدام تكوين خلية جديد، تم تقليل الاعتماد على كاثود الهواء ومع ذلك كانت لخلية الوقود الميكروبية أداء تفريغ أفضل بكثير. تم تقييم النموذج الأولي الذي تبلغ سعته 5 لترات على أنه 5 مللي أمبير، وأظهر قدرة تفريغ تبلغ 2.2 أمبير في الساعة بمتوسط جهد تشغيل يبلغ 0.8 فولت. في النهاية، يعد التكوين الجديد للخلية هو التصميم الأنسب للتوسيع لأنه يتطلب كاثودًا هوائيًا أقل وحجمًا أصغر وقدرة حجمية أقل.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Materials Engineering).

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DECLARATION

I hereby declare that the thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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LIST OF ABBREVIATIONS

CPKO	Crude palm kernel oil
CPO	Crude palm oil
EFB	Empty fruit bunches
DET	Direct electron transfer
MET	Mediated electron transfer
MFC	Microbial fuel cell
MMFC	Medium microbial fuel cell
Min	Minute
HPLC	High phase liquid chromatography
LMFC	Large microbial fuel cell
LSV	Linear sweep voltammetry
OPF	Oil palm fronds
OPT	Oil palm trunk
ORR	Oxygen reduction reaction
PC	*Phanaerochate Chrysosporium*
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PEM	Proton exchange membrane
POME	Palm oil mill effluent
PPF	Palm pressed fibers
Sec	Second
SEM	Scanning electron microscope
SMFC	Small microbial fuel cell
TGA	Thermogravimetric analysis
UV	Ultraviolet
WRF	White rot fungus

LIST OF SYMBOLS

e^-	Negatively charged ion
H^+	Hydrogen ion
I	Current
P	Power
R_{int}	Internal resistance
V	Voltage

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Gradual depletion of existing energy source in recent decades, especially of fossil fuels, against the high demand for energy, has shifted research and development towards alternative energy sources such as fuel cell, photovoltaic panels, wind turbines and water turbines (Hayes, 2018).

Fuel cells is a technology of converting chemical energy into electrical energy and divided into two types chemical and biological. Chemical fuel cell converts chemical energy into electrical energy through molar conversion of reactants with or without the presence of chemical catalysts, whereas biological fuel cell utilizes the fidelity of microorganisms as biocatalysts to convert chemical energy to electrical energy (Ghassemi & Slaughter, 2017).

There are two types of biological fuel cells, which are enzymatic fuel cell and microbial fuel cell (MFC) (Palmore & Whitesides, 1994). Enzymatic fuel cell used oxidoreductase enzymes as an electrocatalyst at the anode and cathode instead of metallic electrocatalysts (Atanassov et al., 2007). Oxidoreductase enzyme is an enzyme that can catalyze oxidation and reduction reactions. While, MFC used microorganisms such as fungi or bacteria either at anode or cathode extracting electric current from organic waste (Pant et al., 2010).

Recent research have focused on deriving energy using waste from agriculture; EFB and POME from the palm oil industry are used to produce electricity using MFC. By using waste, not only it can be used as a substrate for the fuel cell but also reduce waste accumulation.

Bacterial MFCs were more investigated as compared to fungal ones. In fungal MFCs, yeasts has been the most widely employed microbes because they are easy to grow and susceptible to biological and genetic manipulation (Sekrecka-Belniak & Toczyłowska-Maminska, 2018; Goswami & Mishra, 2018). Recent developments in the MFC technology have pushed the maximum power density in the watt-range (Liu et al., 2020; Flimban et al., 2019). However, the technical and economic viability of the technology are still far from the sustainability criteria (Li et al., 2014). This work proposes a hybrid microbial electrochemical system that simplifies the complexity of the bioelectrochemical system design and therefore its cost, while at the same time enhances the energy output markedly, paving the way towards a high energy gain yields. Further, although it is a microbial system, with the proper choice of microbes and substrate, the hybrid system is able to function in a passive mode i.e. operating in uncontrolled ambient surrounding under prolonged duration (more than one month).

This system employs the naturally occurring white-rot fungi i.e. *Phanaerochaete chrysosporium* fungus (Basidiomycete). This fungal strain able to degrades lignin and other biopolymers by producing ligninolytic enzymes such as laccase (Paramjeet et al., 2018; Baldrian, 2006). Laccase is an oxidoreductase that belongs to the copper-containing enzyme family that demonstrates specific affinity for oxygen as their electron acceptor. The fungal microbes were cultured in carbohydrate-based electrolyte and fed with oil palm empty fruit bunch (EFB) as the organic substrate. As the fungal microbes degrade the lignin-rich waste (EFB), the laccase concomitantly catalyses the reduction of molecular oxygen. Therefore, coupling an electropositive metal anode such as zinc and an air electrode in an enclosure filled with fungal microbes fed with lignin-rich agrowaste constitutes a bioelectrochemical cell. In essence, the bioelectrochemical system is a microbial zinc/air cell that degrades waste as it generates electricity.

1.2 PROBLEM STATEMENT

Bioelectrochemical system has long been regarded as the nature's solution in the quest for sustainable energy substitutes for fossil fuels. Its low energy gain yields i.e. low-output/high-cost, however, continues to be a formidable challenge to date. Enzymatic fuel cell possesses a much higher energy density than the microbial fuel cell. However, since enzymes are functional proteins that degrade over time, they must be replenished over time and hence added cost to the existing expensive system. In microbial system, on the other hand, the living microbes continuously produce enzymes as long as they are fed and thus the system possesses extended lifetime.

However, widespread use of MFC technologies are currently being stumbled by expensive components such as the membrane separator needed in dual chamber MFC, and also various controlling devices since the living microbes need to be properly and consistently maintained to ensure their growth and reproduction (Logan et al, 2015). Typically, a bioelectrochemical system comprises of dual chamber with a semi permeable polymer such as cellulose which separates the anodic and cathodic compartments. The membrane separator allows the transfer of ions from anode to cathode while preventing diffusion of oxygen from cathode compartment. The membrane, however, is costly and will add to the operation cost. Another downside is the membrane will increase the internal resistance of the cell and hence will reduce further the low power output of the system.

Terminal electron acceptor also affect the performance of MFC. Commonly used electron acceptors are nitrite, oxygen or dissolved oxygen and ferricyanide. When selecting the terminal electron acceptor, the availability and sustainability must be considered. In laboratory scale, ferricyanide is commonly used but it is not feasible when the MFC need to be up scaled due its high toxicity and non-recyclable (Ucar & Angelidaki, 2017). While, oxygen or to be more exact dissolved oxygen (DO) is a more practical choice in both single and double chamber MFC since it is abundant and have a high redox potential. The downside of using oxygen is its lower rate of oxygen reduction when carbon electrode is used, but it can be solved by using catalyst or biocatalyst (Commault et al., 2017).

Aside from that, scaling up microbial fuel cell also have several issues such as, high irreversible losses which caused activation, mass transfer and ohmic losses. High losses eventually lead to low power output thus limit its application (Koroglu et al., 2018). Other than that, high production cost including material, design, component and fabrication cost also need to be considered (Koroglu et al., 2018). These two limitations can be overcome by designing a novel configuration which can lower both limitations.

Therefore, self-sustainability and inherent issues of cost and system complexity remain as daunting challenge for practical implementation of MFCs. The present work is dedicated towards the development of low cost, stand-alone and self-sustaining MFC from fungal degradation of EFB agrowaste.

1.3 IMPORTANCE OF STUDY

Regardless of bioelectrochemical system components and design, the contemporary interest is to utilize it in converting waste i.e. complex organic substrate into non-polluting, benign by-products as it produces energy. Extensive efforts have been directed towards wastewater treatment in particular (Li et al., 2016; Palanisamy et al., 2019). This work focuses on agrowaste from palm oil industries. Malaysia is among the largest producers of palm oil i.e around 85%. Out of the fresh fruit bunch that is normally harvested for processing, 90 percent of the oil palm production masses are destined for waste. This highlights the vast accumulation of oil palm waste. Using fungal biofuel cell, the biomass wastes can be degraded into environmentally benign residues concurrently utilized for the production of clean and renewable energy.

1.4 OBJECTIVES

The present study is carried out to achieve the following objectives:

i. To develop a microbial zinc-air cell employing white rot *P. chrysosporium* fungus as the source of biocatalyst for the oxygen reduction.

ii. To identify the best configuration for maximum power output and discharge capacity from the microbial zinc-air cell.

1.5 RESEARCH METHODOLOGY

This research was carried out based on the following approaches:

1. MFC Design – A hybrid biofuel cell with single microbes was chosen for the following reasons:
    a. reduction in complexity of MFC design and hence the cost
    b. practicality for variety of applications
2. Choice of microbes for the MFC – White rot *Phanaerochaete chrysosporium* fungus is one of the wood degrading microorganisms. White rot fungi excrete laccase which is extensively studied for bioremediation applications. *P. chrysosporium* has been reported to produce more complete lignin degrading enzyme complex than most other strains (Singh & Chen, 2008).
3. Choice of organic substrate for fungal microbes in the MFC – EFB possesses high lignin content which is the natural substrate for white rot fungi. Our palm oil industry generates tremendous amount of waste, among others is EFB biomass. This work is one the initiatives to convert waste into renewable energy.
4. Characterization of lignocellulosic activities of *P. chrysosporium* in the MFC – Indirect approaches were adopted. Observations were made based on MFC electrochemical performance, HPLC analyses, TGA profile, SEM and optical microscopy imaging, rather than using enzyme assays methods which are more costly and time consuming. Besides, there is no general agreement on the lignin degradation mechanisms due to the complex nature of lignin chemical structure
5. Scaling up of MFC – A new cell configuration was proposed.

1.6 SCOPE OF RESEARCH

To achieve the stated objectives, this project can be divided into four parts:

1. Lignocellulosic activities of *P. chrysosporium* on EFB

   Lignocellulosic activities of *P. chrysosporium* were studied using HPLC analysis, LSV, TGA and SEM. The purpose was to correlate the electrochemical performance of MFC with metabolic activities of fungal microbes and finally to propose possible redox reaction mechanisms or pathways.

2. Design, fabrication and characterization of hybrid biofuel cell – microbial zinc/air cell.

   An electropositive zinc anode was paired against an air electrode in an enclosure filled with *Phanaerochaete chrysosporium* fungal microbes fed with lignin-rich agrowaste (EFB) in PDB electrolyte. The aim was to simplify the MFC design yet produces high power output. The MFC was characterized according to polarization profile, galvanostatic discharge and LSV.

3. MFC scaling up

   Several approaches or cell design configuration were studied so as to scale up the MFC.

1.7 THESIS ORGANIZATION

Chapter one of the thesis provides an overview of the work – rationale of study, research scope and methodology. Chapter two presents a brief review on MFC, redox mechanisms, electrodes material, substrates and microbes employed. Chapter three gives the details of methodology and experimental work. Chapter four discusses the results and findings of this work. Chapter five concludes the findings and recommends directions for future work.