UNIVERSITI TEKNOLOGI MARA

BIOENERGY GENERATION FROM LACCASE-GLUCOSE OXIDASE IN SINGLE AND DOUBLE CHAMBER OF ENZYMATIC FUEL CELL

NURRISA BINTI ASRUL

Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science

Faculty of Applied Sciences

MSc

February 2016

ABSTRACT

The present work investigates a glucose oxidase-laccase enzymatic fuel cell (EFC) employing simplified system design – freely suspended enzymes and their respective substrates. The problem with conventional EFC was the complexity of the system that limits its application for e.g the immobilization procedure which was tedious and has an adverse effect on enzyme activity. EFC also had a low performance system. Single chamber (membraneless) and double chamber design are studied. The EFC comprises of a nickel mesh as the oxidative current collector (and also continuously feed oxygen into the system) and a carbon-based air electrode as the reductive current collector, enclosed in acrylic casing of 3 ml volumetric capacity. The anolyte consists of glucose oxidase enzyme (10 U), glucose substrate (200 mM) and FAD co-enzyme (3.8 mM), while the catholyte consists of laccase enzyme (10 U) and syringaldazine substrate (216 μM). Three types of anolyte/catholyte buffer electrolyte are studied – citrate/citrate, phosphate/phosphate and citrate/phosphate, in the pH range 5 – 6.5. A biocatalytic electrochemical system is highly sensitive. Consequently, any variation in the electrolyte formulation would affect the cell discharge performance. Thus, the discharge profile capacity of the EFC is utilize to elucidate the optimum electrolyte formulation. Though the approach is indirect, the observed changes are obvious, suggesting the method is viable. Despite its simple design features, the EFC demonstrated attractive performance characteristics. The single chamber design registered an OCV of 0.96 V, discharge capacity of 1 mAh (rated at 50 μA) and possessed a volumetric energy density of 286 $\mu W/cm^3$. On the other hand, the double chamber EFC design showed better discharge performance with higher operating voltage and an extended discharge duration by 12%. This could possibly be attributed to the occurrence of charge leakage phenomenon in single chamber design since the anolyte and catholyte were freely mixed. These performance characteristics are considered comparable to biocatalytic energy systems employing much more complicated design such as using immobilized enzymes and mediator, multi-walled carbon nanotubes (MWCNT), oxygenated electrolyte etc., as well as operated in controlled environment. In the EFC design, a commercial air electrode is used as the cathodic current collector which also serves as the ambient oxygen diffusion site to continuously feed oxygen into the system. Utilization of air electrode is shown to enhance the cell output by approximately 30.5 %. As the cell was characterized in an open ambient surrounding, the activity decay of both glucose oxidase and laccase were monitored continuously for 5 days. Both activity decay profiles are similar – a rapid drop in the enzyme activity in the first 20 hours (35-40%), followed by rather a steady region (25% activity decline for about 60 hours duration), and ended by a rapid drop in enzyme activity. Under exposure to uncontrolled ambient conditions, the enzymes could last for about 100 hours.

ACKNOWLEDGEMENT

I am grateful to Allah for the good health and wellbeing that were necessary to complete this thesis. I wish to express my sincere and deepest gratitude to my supervisors, Assoc. Prof. Dr. Ab Malik Marwan Ali, Dr. Raihan Othman (IIUM) and Prof. Dr. Muhd Zu Azhan Yahya (UPNM) for their aspiring guidance, insightful comments, considerable encouragements and patience in supervising me to complete this thesis.

I take this opportunity to thank to all iMade Research Group members (Zaidatul Salwa, Hamizah, Fariz, Fairoz and others) and the Faculty of Applied Sciences, UiTM Shah Alam staff (Puan Juhaida, Puan Musliha, Puan Noorizan and others) for their help and support.

I would like to appreciate my research external guides from International Islamic University Malaysia (IIUM), Dr Rashidi Othman (Kuliyyah of Architecture & Environmental Design), Prof Hamzah Mohd Salleh (Kuliyyah of Biotechnology Engineering), Br. Aziz Ahmad, Br. Sharul, Sis Aimi, Dr. Nor Liza, Sis Hanisah, Sis Noraini & Sis Akmal (Energy Research Lab members, Kuliyyah of Engineering) and all the people who provided me with the facilities being required and conductive conditions for this research.

I also place on record, my sense of gratitude to my friend Ili Nadhirah from University of New South Wales (UNSW) Australia and others, who directly or indirectly, have lent their hand in this venture.

This thesis is dedicated to my husband (Muhammad Hazmi), my daughter (Syafina Insyirah) and my parents for the unceasing encouragement, support and attention. Alhamdulillah.

TABLE OF CONTENTS

	Page
CONFIRMATION BY PANEL OF EXAMINERS	ii
AUTHOR'S DECLARATION	iii
ABSTRACT	iv
ACKNOWLEDGEMENTS	v
TABLES OF CONTENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	xi
LIST OF SYMBOLS	xiii
LIST OF ABBREVIATIONS	xiv
CHAPTER ONE: INTRODUCTION	1
1.1 Overview	1
1.2 Justification of Research	3
1.3 Problem Statement	3
1.4 Objectives of Research	4
1.5 Scope of the Study	4
1.6 Significance of the Study	5
CHAPTER TWO: LITERATURE REVIEW	6
2.1 Introduction	6
2.2 Classification of Biofuel Cells	10
2.2.1 Microbial Fuel Cells	13
2.2.2 Enzymatic Fuel Cells	14
2.3 Important Aspects of Enzymatic Fuel Cells	16
2.3.1 Types of Electron Transfer	16
2.3.1.1 Direct electron transfer (DET)	17
2.3.1.2 Mediated electron transfer (MET)	18

CHAPTER ONE
INTRODUCTION

1.1 OVERVIEW

World is currently facing unbalanced energy management and consequently serious environmental issues. By middle of the 21st century, the world population is expected to double from the current population. Hence the demand for energy is expected to increase massively for the next 40 years. Meanwhile, the existing nonrenewable resource energy i.e. the fossil fuel will be depleted within the next 100 years (Larminie *et al.*, 2003). Thus, the development of renewable energy sources is no longer an option for us. Biofuel cell has the potential to be nature's solution to the quest towards a clean and renewable type energy source. Due to the versatile applications in biological fuel cell research such as portable electronic device and environmental monitoring, this enzyme-based electronics have captivated rising interest in this decade.

Biofuel cells (BFC) were first introduced in 1911 when Potter cultured yeast and *Escherichia coli* cells on platinum electrodes and after that during 1962, the enzymatic biofuel cells were invented employing glucose oxidase enzyme to oxidize glucose at anode. Over the last 48 years, many improvements have been made in enzymatic BFC, however compared to conventional fuel cells, the research and development of enzymatic BFC are still considered new. Figure 1.1 indicated source from SciFinder Scholar regarding the Chemical Abstract database keyword search using words "biofuel cell" from literature for the publication from year 1974 until 2010. The drastic increment on the number of publications can be seen from 2000 until 2010 which indicated that the research of BFC is really interesting to explore.

BFC can be categorized under one of fuel cell branch. Basically, fuel cell is an electrochemical device that transforms chemical energy directly to electrical energy as long as the fuel and oxidant are supplied to it. Meanwhile, BFC is considered a subset of fuel cell that employs biocatalyst. There are two types of BFC. The type of biocatalyst employed defines the main types of BFC. Microbial fuel cells (MFC) employ living cells (such as bacterial species) as biocatalysts, whereas the enzymatic