DEVELOPMENT OF ENZYMATIC ZINC-AIR BIOFUEL CELL USING LACCASE

BY

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ABSTRACT

A hybrid biofuel cell, a zinc-air cell employing laccase as the oxygen reduction catalyst is investigated. The bioelectrochemical system combines the well-understood zinc-air cell and an enzymatic biofuel cell - an electropositive zinc element is coupled with the biocatalytic activity of the laccase enzyme. As a result, the biofuel cell design is simplified since the anolyte components (enzyme, substrate, electron mediator and buffer solution) are replaced with a metallic zinc. Based on the zinc-air system, an air electrode is utilized in the cell design. The air electrode serves both as the cathodic current collector as well as to feed ambient oxygen continuously into the system through its porous structure. A single compartment or membraneless cell design is adopted in this work. The cell electrolyte consists of laccase and syringaldazine in potassium dihydrogen phosphate buffer of pH 6.5. Laccase, sourced from *Rhus vernicifera*, serves as the biocatalyst for the electroreduction of oxygen from the ambient air, while syringaldazine acts as the phenolic substrate for laccase. Unlike most biofuel cells, in the present work laccase enzyme is left to be freely suspended in the buffer electrolyte. Besides, the cell is operated under open ambient conditions. The fabricated cell is characterised based on its open-circuit voltage, power density profile and galvanostatic discharge. Various aspects of cell design – the use of air electrode, syringaldazine as an enhancer, charge leakage phenomenon and the use of high surface area electroplated zinc anode, are also investigated. The activity of laccase as an oxidoreductase is clearly prevalent from the cell discharge profiles. The zinc-air biofuel cell registered an open-circuit voltage of 1.2 V and capable to generate a maximum power density of 3.28 mW at 0.4 V and 8.5 mA. The highest cell capacity obtained is 7.5 mAh. Despite its simple design features and operated not under controlled conditions, the hybrid biofuel cell studied demonstrated power output of comparable performance to that of biocatalytic cells utilising a much more complex system design – immobilized enzyme, controlled temperature and humidity, oxygenated electrolyte etc. The results of this work shall serve as a reference for biofuel cell research to evaluate the efficacy of immobilizing enzymes onto electrodes. Furthermore, the developed biofuel system employing freely suspended enzyme is applicable for microbial fuel cell.

خلاصة البحث

في توظف مادةlaccase كمساعد للتقليل أو الحد منالأوكسجين.نتيجة لذلك ،تم وضع تصميم لخليةبسيطةتتكون من:دائرةواحدة منزوعة الغشاء، وناقل الإلكترون غير موصل و laccase معلق بحرية فيالمحلول الكهربائي . وصّفت الخلية بناء على جهد الدوائرالمفتوحةو تشكيل كثافة الطاقةوالتفريغالناتج من استخدام تقنية galvanostaticعند 0.5مللي أمبير .تم ايضا إثبات نشاطlaccaseباعتبارهاoxidoreductase كجزء منملامحأداءالخلية.سجلتخليةالوقود الحيويالزنكالهواءجهدمقداره 1.2 فولت للدائره المفتوحه، وكانت قادرةعلى توليدطاقة قصوى تبلغكثافتها3.28ميلي واطعند 0.4فولت . أخيرا على الرغم من بساطة التصميم لهذه الخلية،فإن انتاج الطاقة منها كان مقاربا لانتاجخلاياbiocatalytic ذات نظامالتصميم الأكثر تعقيدا8.5.مللي أمبير .قدرة أعلى الخلية التي تم الحصول عليها هو 7.5 ماه .على الرغم من ميزاته تصميم بسيط وتعمل في ظل ظروف لا تسيطر عليها، ودرس في خلية الوقود الحيوي أظهرت الهجين انتاج الطاقة من الأداء مماثلة لتلك الخلايا biocatalyticالاستفادة من وجود أكثر من ذلك بكثير تصميم نظام معقد --انزيم يجمد، ودرجة الحرارة والرطوبة تسيطر عليها، الاوكسيجين المنحل بالكهرباءهذه الرسالة تم إستقصاء خليةالوقود الحيويالهجين ،خليةزنكالهواء التي .إلخ الحالي الزنك laccase نظام biocatalyticيوضح أن ملامح جذابة تطويرها.

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 (Biochemical-Biotechnology Engineering).

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I certify that I have 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 (Biochemical-Biotechnology Engineering).

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DECLARATION

I hereby declare that this 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.

Abdul Aziz bin Ahmad

Signature ____________________ Date _____________________

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DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

DEVELOPMENT OF ENZYMATIC ZINC-AIR BIOFUEL CELL EMPLOYING LACCASE AS THE OXYGEN REDUCTION CATALYST

Affirmed by Abdul Aziz bin Ahmad.

____________________ Signature

____________________ Date

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ABDUL AZIZ BIN AHMAD

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ADH	Alcohol dehydrogenase
BFC	Biological fuel cell
BOD	Bilirubin oxidase
CNT	Carbon nanotube
COD	Chemical oxygen demand
DET	Direct electron transfer
EDTA	Ethylenediaminetetraacetic acid
EFC	Enzymatic fuel cell
FAD	Flavin adenine dinucleotide
GDH	Glucose dehydrogenase
GOx	Glucose oxidase
GPES	General Purpose Electrochemical System
KOH	Potassium hydroxide
$K_4Mo(CN)_8$	Potassium octacyanomolybdate, and
$K_4W(CN)_8$	Potassium octacyanotungstate
MDH	Malate dehydrogenase
MEMS	Microelectromechanical system
MET	Mediated electron transfer
MFC	Microbial fuel cell
NAD	Nicotinamide adenine dinucleotide
NASA	National Aeronautics and Space Administration
OCV	Open circuit potential
ORR	Oxygen reduction reaction
PQQ	Pyrroloquinolinequinone
RT	Room temperature
SEM	Scanning Electron Microscope
XRD	X-ray diffraction

LIST OF SYMBOLS

C	Discharge capacity
E	Electromotive force
I	Current
P	Power
Q	Energy
R	Resistance
e-	Negatively charged electron
H^+	Hydrogen ion
Hg^{2+}	Mercuric ion
V	Voltage

CHAPTER ONE

INTRODUCTION

1.1 OVERVIEW

Research on new and renewable source of energy is attracting the attention of researchers all over the world. One of the emerging fields of research in response to this is the research and development of fuel cells. A fuel cell is an electrochemical device that continuously converts chemical energy to electrical energy for as long as fuel and oxidant are supplied to it. Interesting offspring of fuel cell technology and biotechnology are biofuel cells. These devices employ biological catalysts for the oxidation and reduction processes of fuel source. Biological fuel cells (biofuel cells) are defined as fuel cells that rely on enzymatic catalysis for at least part of their activity (Palmore and Whitesides, 1994). Main types of biofuel cells depend on biocatalysts used. Microbial biofuel cells (MFC) employs whole living cells (microorganisms) whereas enzymatic biofuel cells (EFC) employs enzymes (functional proteins) (Minteer et al., 2007). EFCs have several positive attributes with respect to energy conversion, namely, the use of renewable catalysts, ability to operate at room temperature and physiological pH, and possess much higher power densities as compared to MFC. In addition, enzymes have the added advantage of catalysing specific and defined reactions (Bullen, 2006; Bond, 2005).

In the present work, a hybrid biofuel system is studied - an enzymatic zinc-air cell. Zinc-air cell belongs to metal-air electrochemical system in which the electropositive metal anode is coupled with the electronegative oxygen of the ambient air. The utilization of the electronegative reactant from the ambient air, which is free and unlimited, is the main attractive feature of metal-air electrochemical system.

However, catalyst is required to accelerate the oxygen reduction reaction (ORR) at the air cathode. Typical catalyst employed includes transition metal compounds such as oxides and organometallic complexes, noble metals and their compounds, and mixed metal compounds including rare earth metals (Vincent et al., 1997). This work explores the use of laccase enzyme in the biocatalytic reduction of ambient oxygen in zinc-air electrochemical system.

Laccase is an oxidoreductase that belongs to the copper-containing enzyme family. This enzyme demonstrates a specific affinity for oxygen as their electron acceptor. It is widely applied in wide variety of industrial oxidative processes such as bioremediation, delignification, dye or stain bleaching, plant fibre modification, biosensors, biofuel cells, ethanol production, etc (Alcalde, 2007). The use of this enzyme is attractive because of its ability to reduce molecular oxygen to form water (Thurston, 1994).

1.2 PROBLEM STATEMENT AND ITS SIGNIFICANCE

The ever increasing global energy consumption and as well as growing concerns on global warming and environmental pollution, leaves us with no alternative but to research into renewable energy source. Biofuel cell is a potential clean energy substitute for fuel cells. The limitations hindering fuel cell technology is complemented by biologically catalyzed fuel cells. It is a clean, non-polluting, renewable energy source and releases benign by-product as it produces energy. This work aims to develop a hybrid biofuel cell as a nature's solution in producing clean and renewable type of energy generation. One of the major fields of research in towards preserving the environment is the research and development in biofuel cells (BFC) as a clean energy substitutes for fossil fuel. The BFC system, however, is rather complex. The anolyte and catholyte of a BFC comprises of specific organic fuel or substrate, biocatalyst and also electronophores or electron transfer mediators. Besides, each anolyte and catholyte requires a unique buffer condition for its optimum condition. Therefore, replacing the anolyte of a BFC with an electropositive metal i.e. zinc metal in this context, reduces the complexity of the system, simplifies the design and makes the handling of the cell easier.

1.3 RESEARCH OBJECTIVES

The main objectives of this work are:

i) To develop an enzymatic zinc-air cell utilizing zinc metal as the anode and laccase enzyme as the biocatalyst for the oxygen reduction.

ii) To identify the electrolyte formulation for the enzymatic zinc-air cell.

iii) To evaluate the electrochemical performance characteristics of the enzymatic zinc-air cell.

1.4 RESEARCH METHODOLOGY

This research is carried out by the following approaches as illustrated in Figure 1.1:

i) Design and fabrication of an enzymatic zinc-air cell/hybrid biofuel cell.

ii) The cell comprises of zinc metal anode, a commercially available carbon based air cathode as the oxygen electrode, buffer solution as the electrolyte and the medium for cathodic catalyst i.e. laccase enzyme. The electrodes, laccase and buffer electrolyte are encapsulated in a home-made acrylic board casing. A membraneless, single chamber design is employed. The goal is to simplify the cell design and maintaining a high power output.Electrochemical performance characterizations

The fabricated biofuel cell is characterized according to its open circuit potential, power density profiles, polarization curves and galvanostatic discharge capability. All measurements are carried out by an Eco Chemie Autolab potentiostat/galvanostat (Model PGSTAT302N-Utrecht, The Netherlands) with General Purpose Electrochemical System (GPES) version 4.9 software. All experiments are performed at room temperature.

iii) Laccase stability determination

In order to gauge the lifetime of the zinc-air biofuel cell, laccase enzyme is assayed spectrophotometrically in ambient environment as the cell is intended for ambient operation.

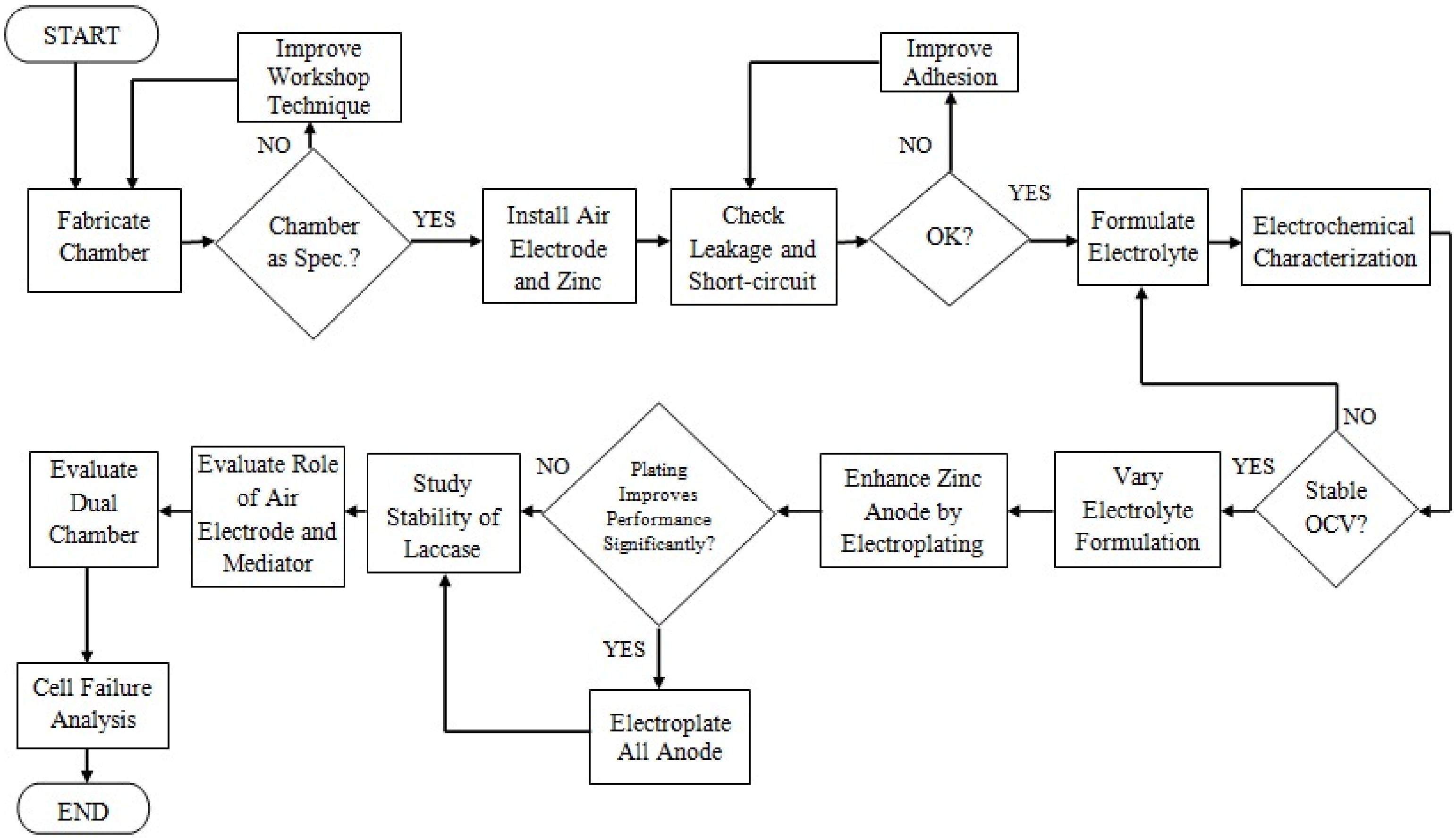

Figure 1.1: Flow Chart of Research Methodology

1.5 SCOPE OF RESEARCH

An enzymatic zinc-air cell is fabricated by coupling a zinc metal anode with a commercially available carbon based air electrode. Laccase enzyme is utilized as the biocatalyst for ambient oxygen reduction. Initial part of the work involves designing and constructing of the liquid cell holder. A single chamber design is employed. The electrolyte formulation is adapted from the work of Ride (1980) which consists of potassium dihydrogen phosphate buffer, syringaldazine substrate and laccase from *Rhus vernicifera.* The assembled cell is characterized according to open circuit voltage, power density and polarization profiles, and discharge capacity. Finally the stability of laccase enzyme is monitored in ambient environment without any external control.

1.6 THESIS ORGANIZATION

Chapter one of the thesis provides the overview of the present work. Chapter two reviews various works, developments and challenges of biofuel cells, recent and past. Chapter two also includes definitions, mechanisms and applications of biofuel cells and biocatalyst used. Chapter three covers the methodology of the experimental work. Chapter four presents the results and discusses the findings obtained. Chapter five concludes the work and suggests the way forward.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

The literature of biofuel cell can be traced back to the very early of twentieth century. It was first reported by Potter (1911) who cultured yeast and *E.Coli* cells on platinum electrodes. Half a century later in 1964, enzymatic biofuel cell was first tested utilizing glucose oxidase and glucose as the biocatalyst and fuel respectively (Davis and Higson, 2007). Deep interest shown by National Aeronautics and Space Administration (NASA) in generating electrical power from human wastes on the space shuttles in the 1960's further fuelled and intensified the investigations on bioenergy generation. Since then many inventions, modifications and improvements have been made in the research and development in bioelectrochemical systems.

The succeeding sections shall highlight fuel cells in general and biofuel cells in particular, properties of the enzyme utilized in this work, key performance characteristics used to evaluate the biofuel cell and applications of biofuel cell. Finally, challenges and recent advancement in biofuel cell are also reviewed.

2.2 FUEL CELLS

Fuel cell is a device capable to directly convert chemical energy to electrical energy. It consists of a cathode, an anode, a conducting electrolyte medium to connect the two electrodes, and an external circuit to consume the electricity. Fuels (reactants) must be supplied to both electrodes as a source for the electron transfer reactions; and catalysts to increase the rate of reaction (Wingard et al., 1982). A typical fuel cell and its major components are as in Figure 2.1. The main difference between a battery and a fuel cell is instead of storing energy, fuel cell converts energy from one form to another and will continue to operate as long as fuels are supplied to it.

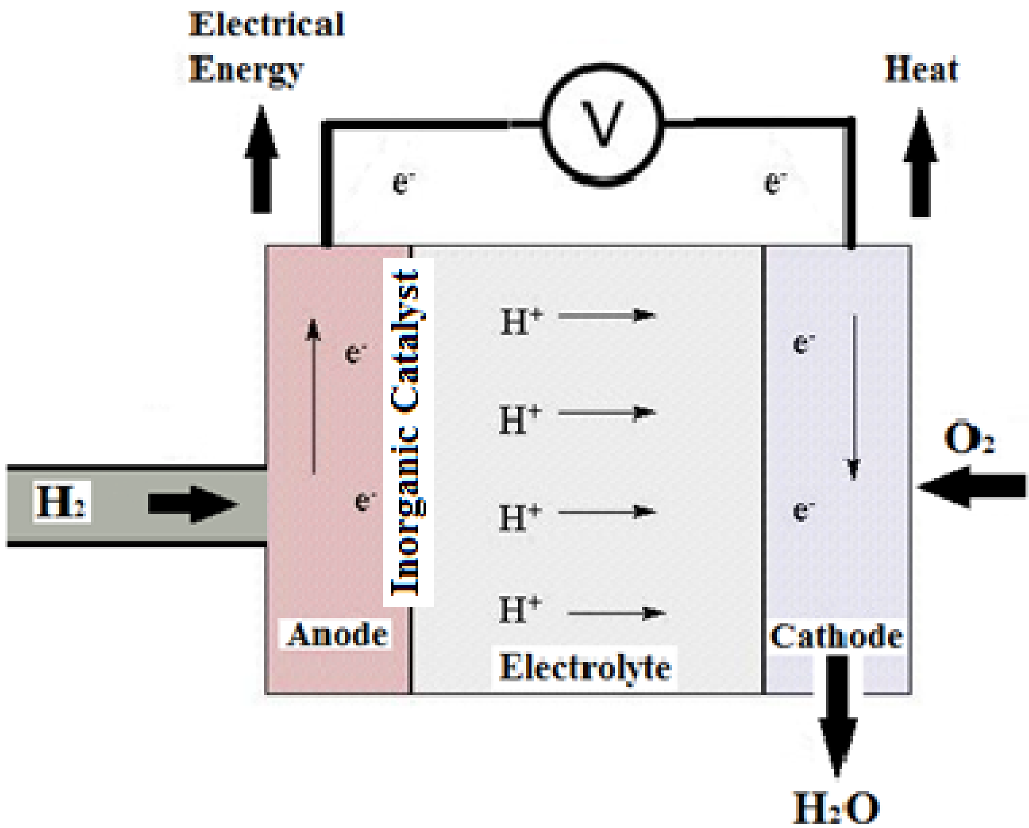

Figure 2.1: Typical fuel cell (www.grc.nasa.gov)

2.3 ZINC-AIR CELL

Zinc-air cell is a subset of metal-air cells which includes lithium-air, sodium-air, magnesium-air and others. In a typical metal-air cell, anode is made from pure metal and the cathode connects to an inexhaustible supply of air (oxygen). Zinc as an anodic fuel has an advantage over other metals due to its unique set of attributes. Among others include zinc's stability in aqueous electrolytes, high specific energy, high volumetric energy density, abundance, low cost, good environmental compatibility, and ease of storage and handling (Linden & Reddy, 2002; Riezenman, 2001). Besides, zinc is also well known for its rapid electrokinetics.

The working principal of a zinc-air cell is similar to conventional electrochemical cells. However, the major difference is instead of having all the necessary reactants inside the cell, zinc-air batteries acquire one of their main reactants (oxygen) from the ambient air (Othman et al., 2001). O_2 molecules enter the electrochemical system through minute holes via a selective membrane and come into contact with a customary porous, carbon-based air cathode. Water and other molecules present in the pores of the electrode react with oxygen molecule to produce hydroxyl via catalytic reaction usually by manganese-based catalyst. These molecules migrate through a separator (membrane) to the zinc metal anode. The hydroxyls bond to zinc ions to form zincate which later on splits into two hydroxyls, zinc oxide and a water molecule. As a result of this process two electrons are released through an external circuit. This continuous flow of electron produces electrical current (Winter & Brodd, 2004).

The overall process can be summarised as follows:

At cathode: $O_2 + 2H_2O + 4e^- \longrightarrow 4OH^-$

At anode: $2Zn \longrightarrow 2Zn^{2+} + 4e^-$

$2Zn^{2+} + 4OH^- \longrightarrow 2Zn(OH)_2$

$2Zn(OH)_2 \longrightarrow 2ZnO + 2H_2O$

2.4 BIOFUEL CELL

Biological fuel cells (biofuel cells) are the progeny of two parent technologies: fuel cells and biotechnology. It is capable of directly converts chemical energy to electrical energy through electrochemical reactions involving biochemical pathways (Bullen et al., 2006). A typical biofuel cell and its key components are shown in Figure 2.2.

Similar to conventional fuel cells, biofuel cells are made of an anode and a cathode usually separated by a selective membrane that only allows positively charged ions. However, unlike conventional fuel cells, biofuel cells utilize enzymatic catalysts, either as they occur in microorganisms, or as isolated proteins rather than precious metals.

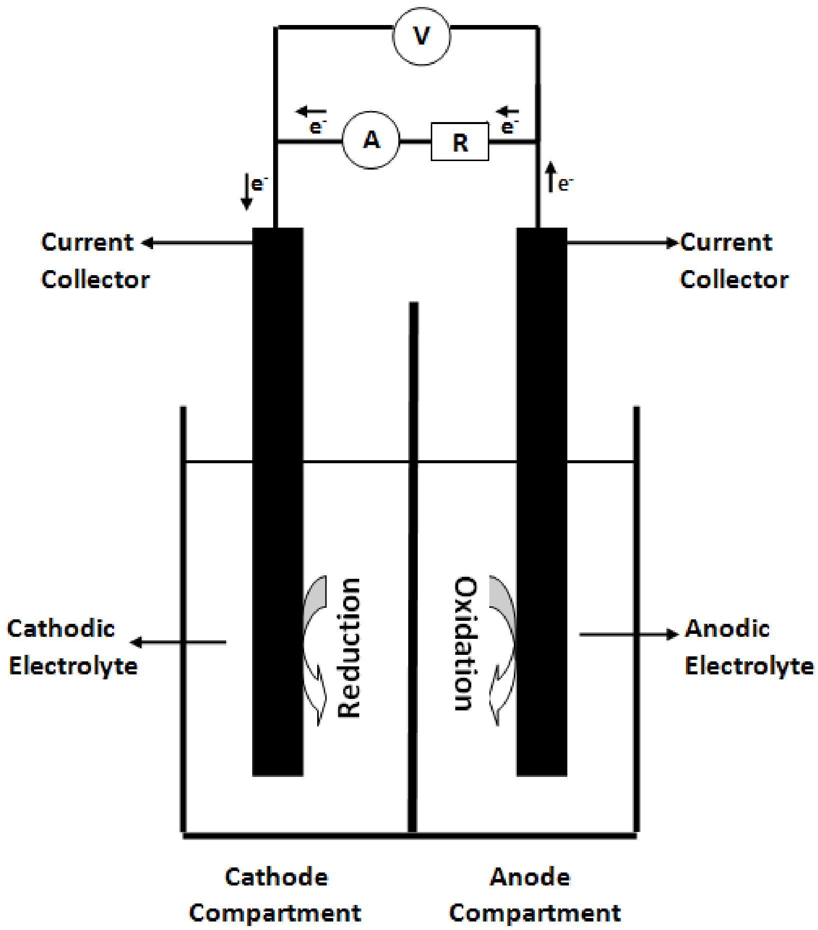

Figure 2.2: Typical biofuel cell employing two sets of biocatalysts

2.5 CLASSIFICATIONS OF BIOFUEL CELL

Electrochemical devices are capable to directly convert chemical or light energy to electrical energy. These devices can be sub-classed into battery, fuel cell and solar cell, as illustrated in Figure 2.3.

Battery is a closed system, whereas fuel cell is an open system. The anodic and cathodic fuels are stored inside the battery and cannot be replaced. On the other hand, the fuels for fuel cells are stored externally and can be replaced (fed continuously).