Helsinki University of Technology - Automation Technology Laboratory
Series A: Research Reports No. 29, December 2006

DEVELOPMENT OF A BIOCATALYTIC FUEL CELL SYSTEM FOR LOW-POWER ELECTRONIC APPLICATIONS

Anja Appelqvist

Dissertation for the degree of Doctor of Science in Technology to be presented with due permission of the Department of Automation and Systems engineering, for public examination and debate in Auditorium AS1 at Helsinki University of Technology (Espoo, Finland) on the 15th of December 2006, at 12 o'clock noon.

Helsinki University of Technology
Department of Automation and Systems Technology
Automation Technology Laboratory

Teknillinen korkeakoulu
Automaatio- ja systeemitekniikan osasto
Automaatiotekniikan laboratorio

Distribution:

Helsinki University of Technology
Automation Technology Laboratory
P.O. Box 5500
FIN-02015 TKK
FINLAND

e-mail anja.appelqvist@tkk.fi
Tel +358-9-451 5147
Fax +358-9-451 3308

© Anja Appelqvist

ISBN-13 978-951-22-8493-1 (print)
ISBN-10 951-22-8493-6 (print)
ISBN-13 978-951-22-8494-8 (pdf)
ISBN-10 951-22-8494-4 (pdf)
ISSN 0783-5477

Picaset Oy
Helsinki 2006

HELSINKI UNIVERSITY OF TECHNOLOGY
P.O. BOX 1000, FI-02015 TKK
http://www.tkk.fi

ABSTRACT OF DOCTORAL DISSERTATION

Author Anja Appelqvist (née Ranta)

Name of the dissertation

Development of a biocatalytic fuel cell system for low-power electronic applications

Date of manuscript September 25, 2006 | Date of the dissertation December 15, 2006

☑ Monograph | ☐ Article dissertation (summary + original articles)

Department Automation and Systems Technology
Laboratory Automation Technology Laboratory
Field of research Biotechnology, energy technology
Opponent(s) Professor Bert Hamelers and Adjunct Professor Matti Korhola
Supervisor Professor Aarne Halme
(Instructor)

Abstract

This thesis describes the development of a biocatalytic fuel cell which is intended for low-power electronic appliances. The operation of a biocatalytic fuel cell is based on the same principles as are metal catalyst fuel cells. The main difference between the two technologies is the catalyst material: in biological fuel cells the catalytic power is derived from a microorganism or an enzyme instead of the usual platinum-based transition metal catalysts. Activity in connection with biocatalytic fuel cells has been lively during the past decade, as has been the study of fuel cells in general. In principle, biocatalytic fuel cells offer inexpensive catalyst and component materials in contrast to metal catalyst fuel cells. However, so far the performance values of the metal catalyst fuel cells have been at least 10 times better. The essence of this study is the analysis of restrictions on electron transfer in the energy generation process of a biocatalytic fuel cell, with the emphasis being placed on the anodic process. Consequently, methods to improve functionality in respect of power and current density will be discussed.

The novel Direct Methanol Biocatalytic Fuel Cell (DMBFC) that was developed was utilised as a case to study biocatalytic energy generation. The operating principle of the DMBFC is the enzymatic breakdown of methanol by methanol dehydrogenase (MDH) from Methylobacterium extorquens. A significant increase in performance was achieved by improving the stability of the mediator, which enhances the electron transfer rate, and by introducing potassium permanganate as the terminal electron acceptor. Additionally, a first prototype of a stack-structured DMBFC was constructed and tested. Further development is required to optimise the process technology of the stack.

As a result of the research work and development of the DMBFC power source, knowledge of biological energy generation processes has deepened significantly. In addition, a novel method of fuel cell control was created. The power source that was developed, the DMBFC, is the basis for further research towards small disposable power sources based on biocatalytic energy generation.

Keywords biofuel cells, methanol dehydrogenase, methanol, DMBFC

ISBN (printed) 951-22-8493-6 | ISSN (printed) 0783-5477
ISBN (pdf) 951-22-8494-4 | ISSN (pdf)
ISBN (others) | Number of pages 99

Publisher Helsinki University of Technology, Automation Technology Laboratory

Print distribution Helsinki University of Technology, Automation Technology Laboratory

☑ The dissertation can be read at http://lib.tkk.fi/Diss/

TEKNILLINEN KORKEAKOULU PL 1000, 02015 TKK http://www.tkk.fi	VÄITÖSKIRJAN TIIVISTELMÄ

Tekijä Anja Appelqvist (os. Ranta)

Väitöskirjan nimi

Biokatalyyttisen polttokennojärjestelmän kehittäminen virtalähteeksi matalatehoisiin elektronisiin laitteisiin

Käsikirjoituksen jättämispäivämäärä 25.9.2006	Väitöstilaisuuden ajankohta 15.12.2006
☑ Monografia	☐ Yhdistelmäväitöskirja (yhteenveto + erillisartikkelit)

Osasto	Automaatio- ja systeemitekniikan osasto
Laboratorio	Automaatiotekniikan laboratorio
Tutkimusala	Biotekniikka, energiateknologia
Vastaväittäjä(t)	Professori Bert Hamelers ja Dosentti Matti Korhola
Työn valvoja (Työn ohjaaja)	Professori Aarne Halme

Tiivistelmä

Väitöskirja käsittelee matalatehoisten elektronisten laitteiden virtalähteeksi tarkoitetun biokatalyyttisen polttokennon kehitystyötä. Kyseisen polttokennotyypin toiminta perustuu samoihin periaatteisiin kuin metallikatalyyttipolttokennojen. Pääasiallisin ero on käytetyssä katalyyttimateriaalissa, joka on ensimmäisessä biokatalyytti (so. mikro-organismi tai entsyymi) ja jälkimmäisessä platina tai platinapohjainen seos. Viimeisen vuosikymmenen aikana biologisiin polttokennoihin liittyvä tutkimus on ollut hyvin vilkasta, kuten polttokennotutkimus yleisestikin. Periaatteessa biologiset polttokennot tarjoavat mahdollisuuden kustannussäästöihin katalyytti- ja rakennemateriaalien suhteen metallikatalyyttiä käyttäviin polttokennoihin verrattuna. Tällä hetkellä biopolttokennojen suorituskyky on vasta noin kymmenesosa perinteisten polttokennojen arvoista. Tämän tutkimuksen ydinaines onkin biologisessa polttokennossa tapahtuvan energian tuottamisprosessin analysointi: elektroninsiirtoa vaikeuttavien tekijöiden, erityisesti anodireaktiossa, sekä toimintaa parantavien menetelmien selvittäminen.

Tutkimuksessa kehitettiin uudentyyppinen biokatalyyttinen suorametanolipolttokenno (DMBFC), jota käytettiin esimerkkitapauksena. Biokatalyyttinen energiantuotanto perustuu tässä tapauksessa metanolin entsymaattiseen hajottamiseen metanolidehydrogenaasin (Methylobacterium extorquens) avulla. Biopolttokennon toimintaa kyettiin parantamaan merkittävästi parantamalla elektroninsiirtoa nopeuttavan mediaattorin stabiilisuutta sekä ottamalla käyttöön kaliumpermanganaatti terminaaliseksi elektronin vastaanottajaksi. Tutkimuksessa kehitettiin myös ensimmäinen prototyyppi DMBFC-periaatteella toimivasta kahdeksan yksikkökennon kennostosta. Kennostorakenteen prosessitekniikan optimointi edellyttää vielä jatkokehitystä.

Tutkimustyön tuloksena on saatu merkittävästi lisätietoa DMBFC-periaatteella toimivan virtalähteen energian tuotantoprosessista sekä uusi tapa ohjata polttokennoa. Kehitettyä biologista polttokennoa ja tutkimustuloksia voidaan käyttää hyödyksi biokatalyytteihin perustuvien kertakäyttöisten virtalähteiden jatkokehityksessä.

Asiasanat biopolttokennot, metanolidehydrogenaasi, metanoli, DMBFC

ISBN (painettu)	951-22-8493-6	ISSN (painettu)	0783-5477
ISBN (pdf)	951-22-8494-4	ISSN (pdf)	
ISBN (muut)		Sivumäärä	99

Julkaisija Teknillinen korkeakoulu, Automaatiotekniikan laboratorio

Painetun väitöskirjan jakelu Teknillinen korkeakoulu, Automaatiotekniikan laboratorio

☑ Luettavissa verkossa osoitteessa http://lib.tkk.fi/Diss/

PREFACE

This research work was carried out in the Automation Technology Laboratory at Helsinki University of Technology (TKK) as part of the Tekes-funded research projects Presto (2000-2001) and Improvement of the performance of fuel cells (PSP) (2001-2003). In addition, Graduate School of Silicon Technology and Microsystems together with the TES and SKS foundations are thanked for the personal grants they provided.

I want to express my gratitude to Professor Aarne Halme, head of the laboratory and supervisor of my thesis work, for his patience, advice, and support in this lengthy process. Dr.Sc. Xia-Chang Zhang has played an essential role in the project. His wide expertise in the field of biotechnology and his practical skills have been an inspiration to me. Doctor Zhang, thank you for the introduction to biofuel cells and your altruistic support in the practical research work. Our electronics expert Sami Kielosto has been an indispensable help and fun to work with. It was interesting to explore the world of business and a start-up company with Dr.Sc. Jussi Suomela. Tomi Ylikorpi is thanked for sharing his expertise in mechanics design. I am grateful to Professor Arto Visala for his advice in finalising the manuscript. Sami Ylönen and Ilkka Leppänen, thank you for the peer support; you really made a difference. I want to thank everyone in the Automation Technology Laboratory for the relaxed atmosphere and for it being an enjoyable place to work. I thank Adjunct Professor Matti Korhola and PhD Rolf Rosenberg for their pre-examination of my thesis and good comments. Professor Bert Hamelers and Adjunct Professor Matti Korhola, I appreciate the time and effort you have spent examining the thesis and debating its contents.

I am deeply indebted to my family and friends for their care and support. I am grateful to my parents for creating a happy childhood and youth spiced with the "spirit of Poli" (Poli = TKK). My brothers Janne, Jouni, and Henri, and my sister Katja, together with their respective families, I am thankful for having you in my life. I have been blessed with absolutely superb grandparents and godmother Anu. The enjoyable times spent with friends have provided a well-needed balance to the work; Minna, Hanna, Kirsi, Tuula, and Vuokko especially, thank you for being the lovely people you are! Last but not least, my very special thanks go to Peci, a role model in many areas of life and an inspiration to me. At the end of the day, this is what life is all about: family and friends.

This thesis is dedicated to the preceding generations of my kin who have wandered the corridors of TKK since 1906.

My great-grandfather, Juho Nykänen
My grandfather, Runar Holm
My parents, Anneli and Pekka Ranta, and my godfather, Jouko Ranta

Espoo, December 2006

Anja Appelqvist

TABLE OF CONTENTS

LIST OF SYMBOLS AND ABBREVIATIONS

Abs	Absorbance [nm]
ADH	Alcohol dehydrogenase
AFC	Alkaline fuel cell
Al_2O_3	Aluminum oxide
AldDH	Aldehyde dehydrogenase
Au	Gold
BioFC	Biofuel cell, biocatalyst fuel cell
BOD	Bilirubin oxidase
c	Concentration c = [mol/l] = [M]
c/t	Molar production rate
C	Capacity, C = [Ah]
C_A	Activation capacitance
CH_2O	Formaldehyde
CH_3OH	Methanol
CHOOH	Formic acid
CHP	Combined heat and power
CO	Carbon monoxide
CO_2	Carbon dioxide
CO_3^{2-}	Carbonate ion
COx	Cytochrome oxidase
Ca^{2+}	Calcium ion
Cu	Copper
DFAFC	Direct Formic Acid Fuel Cell
DL	Gas diffusion layer
DMBFC	Direct Methanol Biocatalytic Fuel Cell
DMFC	Direct Methanol Fuel Cell
DoD	Department of Defence
E	Enzyme
E_{OX}	Oxidised enzyme
E_{RE}	Reduced enzyme
E^0	Standard potential at 25 °C, E^0 = [V]
e^-	Electron
η	Efficiency
F	Faraday constant 96485 C/mol
FAD	Flavin adenine dinucleotide
FAFC	Formic acid fuel cell
FDA	Food and Drug Admistration of the USA
FDH	Formate dehydrogenase
Fe	Iron
Fe_2O_3	Iron oxide
GOx	Glucose oxidase
H_2	Hydrogen
H^+	Hydrogen ion, proton
H_3BO_3	Boric acid
HCl	Hydrochloric acid
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HNQ	1-hydroxy-1,4-naphtoquinone
I	Current, I=[A]

I_{CELL}	Current I generated in a fuel cell
I_0	Output current
I_P	Primary current
ICAO DGP	International Civil Aviation Organisation Dangerous Goods Panel
IEC	International Electrotechnical Commission
IPCS	International Programme on Chemical Safety
IU	Unit of enzyme activity: 1 µmol of substrate consumed per minute
K^+	Potassium ion
kDa	Dalton, unified atomic mass (kDa = 10^3 Da)
$K_3Fe(CN_6)$	Potassium ferrocyanide
K_2HPO_4	Potassium phosphate
$KMnO_4$	Potassium permanganate
KOH	Potassium hydroxide
lac	Laccase
λ	Wave length, λ = [m]
LEFC	Liquid electrolyte fuel cell
Li-Ion	Lithium Ion
M_{OX}	Mediator in oxidised form
M_{RE}	Mediator in reduced form
MCFC	Molten Carbonate Fuel Cell
MDH	Methanol dehydrogenase
MEA	Membrane electrode assembly
MeOH	Methanol
MnO_2	Manganese dioxide
MnO_4^-	Permanganate ion
MV	Methylviologen
MW	Molecular weight
n	Amount of substance, n = [mol]
n/t	Production rate
NAD^+/NADH	Nicotine adeninediamine, reduced/oxidised
NaH_2PO_4	Sodium phosphate
NaOH	Sodium hydroxide
NASA	National Aeronautics and space Administration of the USA
NCBE	National Centre for Biotechnology Education
Ni	Nickel
NiCd	Nickel cadmium
NiMH	Nickel methal hydride
NiO	Nickel oxide
O_2	Oxygen
O^{2-}	Oxide ion
OCV	Open circuit voltage
OH^-	Hydroxide ion
Os	Osmium
P	Power, P = [W] and Product (of enzymatic reaction)
pH	Measure of acidity of a solution in terms of activity of hydrogen (H^+)
PAFC	Phosphoric acid fuel cell
Pd	Palladium
PDA	Personal digital assistant
PEM	Polymer Electrolyte Membrane / Proton Exchange Membrane
PEMFC	PEM fuel cell

PES	Phenazine ethosulphate
pK_a	logarithm of acid dissociation constant K_a
PMS	Phenazine methosulphate
POM	Polyoxymethylene
PQQ	Pyrroloquinoline quinone
Pt	Platinum
PTFE	Polytetrafluoroethylene (''Teflon'')
Q	Quantity of electricity Q = [C]
R	Resistance, R = [Ω] and the reduced form of TMPD
R_A	Activation resistance
R_{IN}	Internal resistance
R_Ω	Ohmic resistance
RFID	Radio Frequency Identification
Rh	Rhodium
RMFC	Reformed methanol fuel cell
Ru	Ruthenium
S	Substrate
$S^{\cdot+}$	Oxidised form of TMPD
SCE	Standard calomel electrode
SHE	Standard hydrogen electrode
SOFC	Solid Oxide Fuel Cell
t	Time
t½	Half-life
T	Temperature, T = [°C]
T^{++}	Oxidised form of TMPD
TiO_2	Titanium oxide
TKK	Helsinki University of Technology
TMPD	N,N,N',N',-Tetramethyl-p-phenylediamine
$TMPD_{OX}$	Oxidised TMPD
$TMPD_{RE}$	Reduced TMPD
U	Voltage, U = [V]
U_L	Load voltage
U_0	Output voltage
U_{OCV}	Open circuit voltage
U_P	Primary voltage
V_{CELL}	Voltage V generated in a fuel cell
W_0	Output power
W_P	Primary power
YSZ	Yttria-doped zirconia

Chapter 1

INTRODUCTION

1.1 Background to the study

The principle of fuel cells was invented in the mid-19th century. The first document was published by a German professor, Christian Friedrich Schönbein, in 1839 and in 1845 the first fuel cell generator was constructed by Sir William Robert Grove, who is considered the founder of fuel cell technology (Bossel, 2000). In the early 20th century another British scientist, Potter, described the principle of biological fuel cells. The technology was rediscovered in the 1960s in connection with space exploration programmes.

The trend in consumer appliances is towards power-hungry devices, such as 3G mobile phones, with energy-consuming applications such as a wireless local area network (WLAN) and terrestrial broadband broadcasting, and battery technology is struggling to keep up with the trend. Consequently, the promise of fuel cells with a large energy content when compared to batteries of the same size has become attractive. Presently, a large part of the worldwide research activity in the field of fuel cells is directed towards using mobile and portable fuel cells to replace batteries.

Interest in biological fuel cells has risen at the same pace as the development of metal catalyst fuel cells. The operation of a biocatalytic fuel cell is based on the same principles as that of metal catalyst fuel cells. The main difference between the two technologies is the catalyst material: in biological fuel cells the catalytic power is derived from a microorganism or an enzyme instead of the usual platinum-based transition metal catalysts.

Biofuel cells have been actively studied at the Automation Technology Laboratory at Helsinki University of Technology (TKK) since the early 1990s. The motivation for the research derived from the practical need to find small-sized, lightweight, yet efficient and long-lasting power sources for mobile robots. As an example of the applications, there was a research initiative from industry to develop long-term energy supply systems for deep-sea use. The first biofuel fuel cell set up at the Automation Technology Laboratory was a microbial fuel cell utilising natural sediment flora collected from the coastal sediments of the Baltic Sea as a catalyst (Zhang, 1995; Zhang and Halme, 1997). This microbial fuel system repeatedly showed good stability for long operating periods (see Table 2.8 for the results). The runs lasted for over one month (700 h). Moreover, the reactions were observed to be self-regulating and self-discharge was negligible. An intriguing phenomenon was observed: the power-to-volume efficiency of the microbial fuel cell increased as the anodic volume decreased. Another interesting finding was the ability to monitor the state of the bacteria by monitoring the changes in colour of the broth in the reaction vessel (Halme et al., 1998).

Since the year 2000 the research work has been focused on enzymatic fuel cells because of the expected technical and customer opinion difficulties related to using living microbes in small consumer products. The biofuel cell technology currently being developed at the Automation Technology Laboratory is based on the enzymatic breakdown of methanol with methanol dehydrogenase from *Methylobacterium extorquens*. The cell type was named the Direct Methanol Biocatalytic Fuel Cell (DMBFC) on the basis of its described operating principle.

1.2 Research problem and aims of the study

The research problem of this thesis is to study the factors affecting the power density of the developed biofuel cell and methods to improve its performance, emphasising the anodic processes. Another purpose is to make an overall assessment of the data acquired during the 3-year research period. The novel direct methanol biocatalyst fuel cell developed in the study will serve as the subject of the case study. The nature of the enzymatic catalyst creates certain technological challenges for the realisation of a practical power source. One is the lower power density compared to transition metal catalyst fuel cells. The counterpart of the DMBFC among traditional platinum catalyst fuel cells is the mobile direct methanol fuel cell. At the moment their performance is in the range of 60-100 mW/cm^2. The best reported biofuel cell power density is 5 mW/cm^2 (Akers, 2005). Another important issue in biofuel cell development is the fact that an enzyme has a shorter operational lifetime than a metal catalyst. However, platinum-based catalysts in metal catalyst fuel cells suffer from poisoning caused by the byproducts or impurities in the fuel, such as carbon monoxide. One of the key issues in improving the power output of biofuel cells is to enhance the electron transfer rate.

1.3 Contribution of the thesis

The main contribution of this thesis is the development of the DMBFC and analysis of the characteristics related to the energy generation process therein. The complete pathway from the fuel through every step of the power generation process is addressed in detail in order to find out the limiting factors. Consequently, ways to improve functionality in respect of power and current density are discussed. As a result of the 3 years of research, the improvement in power density was 19-fold (data from 2000 and 2003). The continuous power density achieved with the DMBFC was 285 $\mu W/cm^2$ at an operational voltage of 0.67 V. The short term performance was 2-fold in respect of power density.

1.4 Outline of the thesis

The contents of the thesis are structured as follows:

Chapter 1: A brief introduction to the subject and research aims of the thesis.

Chapter 2. Review of fuel cell technology: the focus of the review is on mobile metal catalyst fuel cells and biological fuel cells; larger-scale fuel cells are described on a general level.

Chapter 3. Description of the direct methanol biocatalytic fuel cell system utilised in the study and of the experiments.

Chapter 4. Power generation in a direct methanol biocatalytic fuel cell is discussed in detail.

Chapter 5. The results obtained in the study are described and analysed.

Chapter 6. The main results are summarised and future prospects discussed.

1.5 Author's contribution within the research group

The research work presented here was carried out during the years 2000-2003 by a group of 2-3 people. The author's main contribution was the overall assessment of the data obtained during the research and development of the DMBFC. Regarding the experimental part of the work, the planning and execution of the experiments regarding the characterisation of the enzyme and of the stack-structured DMBFC were the responsibility of the author. Other experiments described were performed in cooperation with Dr.Sc. Xia-Chang Zhang. The different cell structures of the DMBFC were designed within the research group. A study of the effects of periodical loading was published in the Master's Thesis of Sami Kielosto. The preceding publications of the author are published under the name Ranta.

Chapter 2

REVIEW OF FUEL CELL TECHNOLOGY

2.1 Introduction to fuel cell technology

A fuel cell is an electrochemical energy conversion device. In one of their articles Palmore et al. (1998) defined a fuel cell in the following manner: "A fuel cell functions by the oxidation of fuel at the anode, simultaneous transfer of electrons and protons to the cathode compartment, and the reduction of a second fuel (typically dioxygen) at the cathode. The power output of a fuel cell is a function of the rate of transfer of electrons through an external circuit (I_{CELL}) and the potential difference between the anode and the cathode (V_{CELL})." This definition is valid for acid electrolyte fuel cells utilising hydrogen as their fuel. In other fuel cell types the mobile ion can be other than a proton. Another definition of the fuel cell underlines the difference between a fuel cell and a battery. A battery contains the substances needed for energy generation; secondary batteries allow recharge by reverse energy generation reaction with electricity.

A fuel cell is an open system, i.e. it does not contain energy, and the fuel and oxidant are brought in from outside the device. An ideal fuel cell would function eternally as long as fuel and oxidant were fed in. According to this definition, some of the energy generation devices called fuel cells should be renamed as hybrid fuel cells. One example of such a device is the Zinc air battery or Zinc fuel cell, in which the zinc is the limiting factor. The device presented in this thesis, the closed cathode DMBFC, also falls into the category of hybrid fuel cells, because the cathode is limited by the oxidative reagent, potassium permanganate. Figure 2.1 illustrates the general operational principle and Equations 2.1-2.2 the reactions inside the fuel cell.

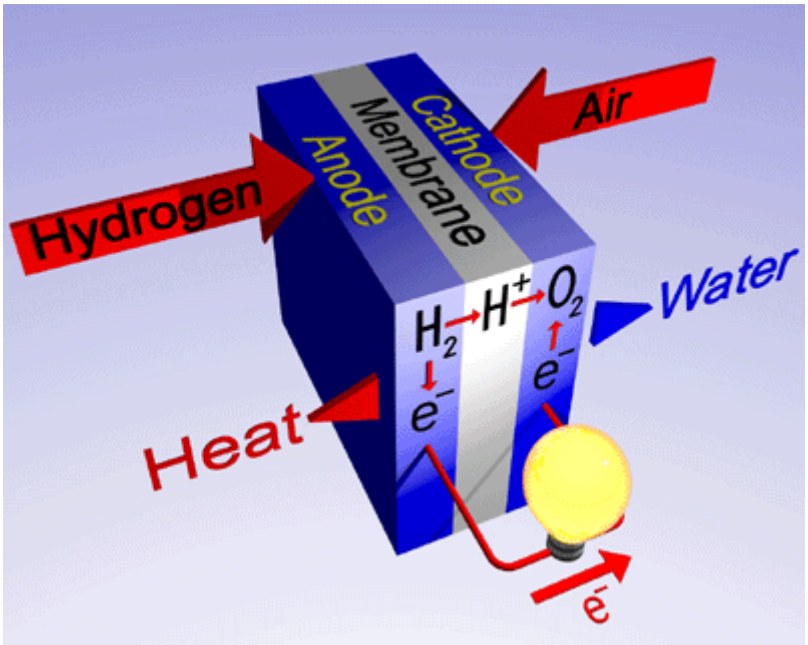


Figure 2.1. Operational principle of a fuel cell (Courtesy of Fuel Cell Today)

Anode: $H_2 \rightarrow 2e^- + 2H^+$ (2.1)

Cathode: $1/2O_2 + 2e^- + 2H^+ \rightarrow H_2O$ (2.2)

$E^0 = 1.23$ V

At the anode electrode hydrogen gas is broken down into protons (hydrogen ions) and electrons. The protons go to the cathode electrode through the proton-conducting membrane separating the two electrodes and the electrons through the external circuit creating electron flow, that is, current. At the cathode oxygen is combined with the protons and electrons and water is formed. The standard potential difference between the electrodes of an $H_2|O_2$ fuel cell is 1.23 V.

In the early days of fuel cell technology, the space exploration programmes had a significant influence on development. At present the military is one of the major financiers in the field. One important driving force in the development of fuel cell technology is the need to find alternative energy generation technologies. The exhaustion of fossil fuels is evident. The western lifestyle and the fast-growing economies in Asia are consuming more and more energy each year. Global warming and the pollution of the environment are forcing the scientific community and governments to push forward new alternative technologies.

As an energy generation technology, fuel cell technology is interesting because the same technology can be applied in many spheres, from industrial-scale energy generation to portable and mobile power sources. The focus of this thesis is on mobile fuel cells, but the other application areas will be briefly described in the following two sections. Figure 2.2 below illustrates the vastness of the application area.

In portable applications the applicable technology is PEM fuel cells (Proton Exchange Membrane or Polymer Electrolyte Membrane). A subspecies of PEM fuel cells is the direct methanol fuel cell (DMFC) technology. PEM fuel cells offer a power generation solution for automotive vehicles, boats, and CHP units (Combined Heat and Power). In the previously mentioned application area (100 W-100 kW), alkaline fuel cells (AFC), solid oxide fuel cells (SOFC), and phosphoric acid fuel cells (PAFC) can be utilised. Appropriate technologies for industrial-scale energy production up to 10 MW are SOFC, PAFC, and molten carbonate fuel cells (MCFC). The basic features of each fuel cell type are described in Table 2.1.

Theoretically, fuel cells are thermodynamically more efficient than combustion engines, the performance of which is restricted by the Carnot cycle. Still, regardless of the potential they embody, there are only a few commercial applications of fuel cell technology. In vehicle and large-scale applications the utilisation of fossil fuels is more cost-effective. On a mobile and portable scale, not all the technological challenges have yet been solved. Additionally, sociological and logistical issues require attention. The different fuel cell types are briefly explained in the following few pages.

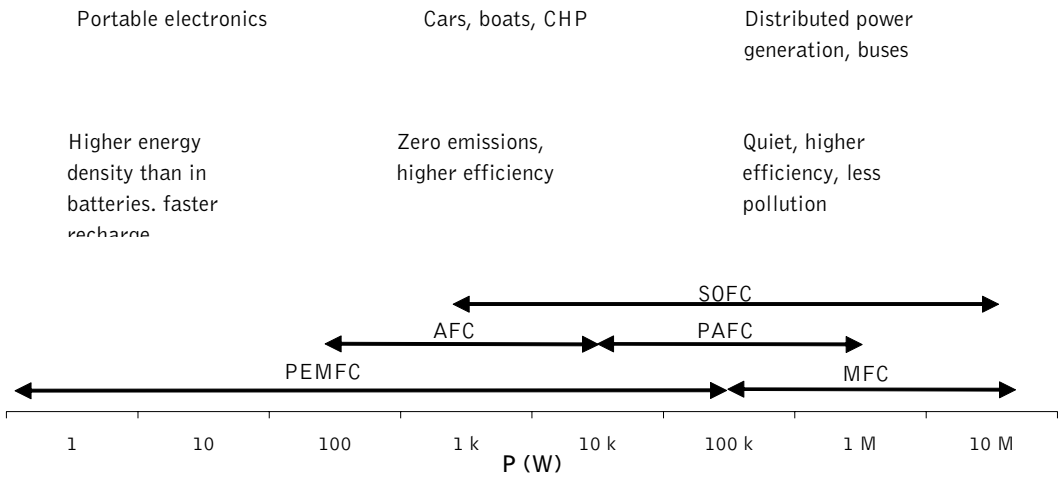


Figure 2.2. Different types of fuel cells, their power ranges and applications. (Adapted from Larminie and Dicks, 2001).

Table 2.1. Principal fuel cell types and characteristics. (Adapted from Larminie and Dicks, 2001*)*

Fuel cell type	Mobile ion	Operating Temperature	Operating pressure	Fuel (anode/cathode)
AFC	OH^-	50-200 °C	2-45 bar	H_2 / O_2 or air
PEMFC	H^+	50-100 °C	1-3 bar	H_2 / O_2 or air
PAFC	H^+	~220 °C	1-10 bar	Natural gas/O_2 or air
MCFC	CO_3^{2-}	~650 °C	1 bar	Hydrocarbon, methane/O_2 or air
SOFC	O^{2-}	500-1000 °C	1-13 bar	H_2, Natural gas/ O_2 or air

The Polymer Electrolyte Membrane fuel cell (PEMFC) was used on the first manned spacecraft of NASA. It can be used in applications from 1 W to 100 kW. The electrolyte is a solid polymer with high proton conductance, and the dominant material is Dupont's Nafion, which is sulphonated polytetrafluoroethylene (PTFE). The catalyst material is platinum or a platinum alloy; the platinum load nowadays is 0.2 mg/cm^2 or lower. Platinum is sensitive to CO poisoning, but the addition of other metals, such as ruthenium, increases the tolerance.

The core of the PEMFC is the membrane-electrode assembly (MEA, Fig. 2.3). Both electrodes are "glued" together with the polymer electrolyte. One critical issue is the water management of the cell. The membrane requires moisture to function but excess water at the cathode causes flooding, i.e. the contact of the gas and catalyst is impaired by too much water. A variant of the PEMFC is the direct methanol fuel cell (DMFC), where the methanol fuel is fed directly into the anode instead of first being reformed. DMFCs are an essential part of small fuel cells and will be discussed later on.

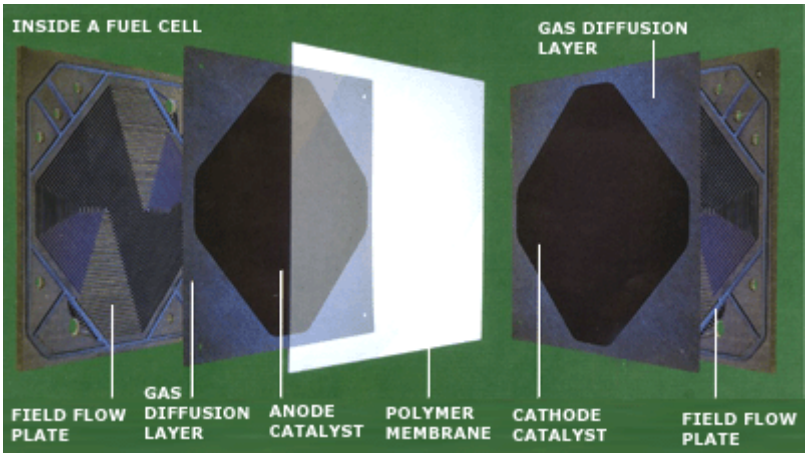


Figure 2.3. Schematic of a Membrane-Electrode Assembly (Courtesy of Fuel Cell Today).

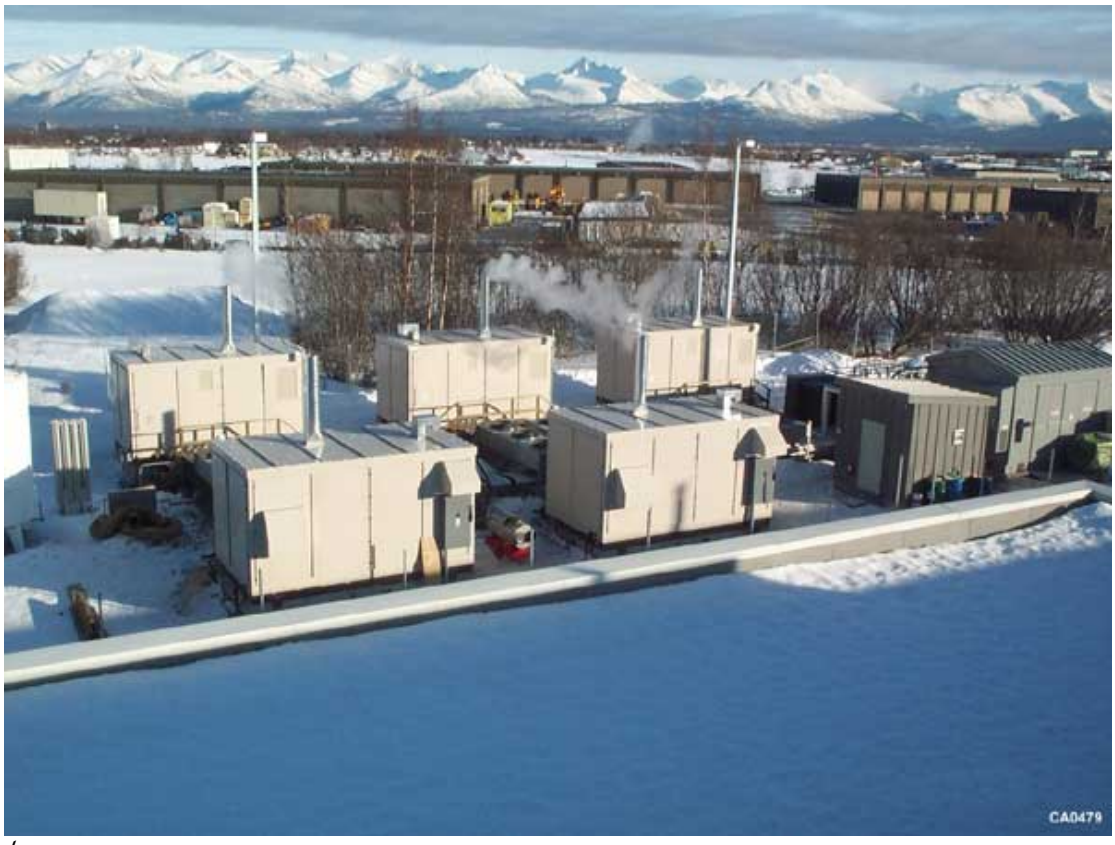
The principle of an alkaline fuel cell (AFC) was described by Sir William Grove in the 19th century (Bossel, 2000). There was an alkaline fuel cell on board the Apollo spacecraft (Fig. 2.4). Alkaline fuel cells are considered to be low-temperature fuel cells; their operating temperatures vary between 50-200 °C. In alkaline conditions the mobile ion is a hydroxide ion, which travels from the cathode to the anode. At the cathode oxygen, water and electrons regenerate OH^- ions. The fuel, hydrogen, together with hydroxide ions, forms water and electrons at the anode. The electrolyte is potassium hydroxide (KOH). The electrolyte can be free or fixed in a matrix.

Figure 2.4. The AFC unit of the Apollo spacecraft, as displayed in the Smithsonian Institute Air & Space Museum; height approximately one metre.

The benefit of the AFC is that the activation overvoltage at the cathode is lower than in fuel cells operating in acidic conditions. Additionally, the materials for the electrodes can be non-noble metals (Ni for the anode and NiO for the cathode). The main problem when operating on the Earth is atmospheric carbon dioxide. It reacts with the alkaline electrolyte, forming carbonate, and subsequently reducing the OH^- concentration and thus the reaction rate. The same phenomenon is stronger if the hydrogen is reformed from hydrocarbons.

Phosphoric acid fuel cells (PAFC) have long been the only commercial fuel cell type. The dominant application is in small-scale electricity and heat generation with CHP units for remote or off-the-grid locations. For example, the UTC Power company has sold around 300 CHP units worldwide (UTC, 2006). The main benefit is the reliability of the system.

a.



b.

Figure 2.5. a. The PureCell Commercial Fuel Cell Power System - Model 200 from UTC Power (200 kW); b. The PureCell station in Anchorage, USA (Courtesy of UTC Power).

The operating principle of the PAFC is similar to that of the PEMFC. The catalyst on both sides is platinum or a platinum alloy. The electrolyte, however, is phosphoric acid (100%). Phosphoric acid is tolerant to CO_2 and has a low freezing point (-2°C at 100% concentration). As a result of its fluidistic properties, the capillary forces keep it inside the matrix material, which consists of silicon carbide and PTFE. The electrodes are also gas diffusion electrodes, as in the PEMFC. The reaction generates heat, which is usually removed with a water-cooling system. If the fuel has too high a sulphur concentration, the poisoned anodes can be regenerated at a high temperature or at a high polarisation potential.

The molten carbonate fuel cell (MCFC) is considered to be a high-temperature fuel cell (T = 600-700 °C). The catalyst materials in this case are nickel and nickel oxide at the anode and cathode, respectively. The electrolyte material is a combination of molten alkali metal carbonates in a ceramic matrix. The mobile ion is carbonate (CO_3^{2-}). In other fuel cells CO_2 is unwanted, but in the MCFC it is needed as the source of carbonate ions. Carbon dioxide is usually collected from the anodic reaction and recycled to the cathode. Carbon monoxide can also be utilised as a fuel, in contrast to most other fuel cell types, in which it is a poison. The high operating temperature makes possible inexpensive catalyst materials and internal reforming, fuel flexibility, and higher overall efficiency, but it also brings with it corrosion issues related to the structural materials used.

The solid oxide fuel cell (SOFC) is the other high-temperature fuel cell type. As the name implies, it is an all-solid device. The catalyst materials are non-noble metals; the anode is made of zirconia cermet containing nickel and the cathode of electronically conducting oxides or ceramics. The electrolyte is yttria-doped zirconia (YSZ). The mobile ion in this type is an anion, O^{2-}. As in the previous type, the high temperature is challenging for the materials.

2.2 Small fuel cells

2.2.1 Introduction

It is first necessary to define the meaning of the terms "small fuel cell", "portable", and "mobile" in this context. The concept of a small fuel cell covers both the mobile and portable fuel cells. In this study a mobile fuel cell denotes a power source which is intended for handheld devices with a power range of 1-20 W. A portable fuel cell is an appliance a person is able to carry, such as a spare power source or back-up power or some recreational power sources having a power range of 20 to about 65 W.

Table 2.2. Division of small fuel cells into mobile and portable ones.

Category	Power range	Application area	Products/examples
Mobile	1-20 W	- mobile phones - laptops - MP3 players - PDA devices	Mobion (MTI microfuel cells)
Portable	20-65 W	- back-up power - recreational power source (boats, mobile homes, etc)	EFOY (Smart Fuel Cells GmbH)

Research in the area of small fuel cells has been lively during the past ten years. Throughout the fast development of handheld electronic appliances, traditional battery technology has so far been able to keep up with rising energy demand. Present-day laptop batteries can last for 5 hours and a regular mobile phone works for one week with one recharge. The yearly capacity increase in battery technology has been 5-10%, but at the moment there is no major technological leap in sight. Having said that, research into novel materials is ongoing and battery technology has the advantage of being a mature and well-adopted technology. An apt remark was once made at a conference: "fuel cells are widely discussed and batteries widely used".

Nevertheless, recently more advanced mobile phones with power-consuming applications (digital video broadcast, wireless Internet access) have increased their power demand drastically. These devices have been aptly nicknamed power-eaters. The demand has risen to such a high level that fuel cells with a higher energy content have started to look more appealing. The general opinion seems to be that fuel cells can more probably fill the power gap than battery technology can. Nevertheless, fuel cell technology still has some important technical issues to solve before it becomes mainstream. Most handheld devices must be redesigned to ensure access of oxygen to the cathode. Some critics wonder how adequate oxygen access can be guaranteed in the everyday use of a mobile phone. People keep their mobile phones in closed places, such as pockets and bags, or lying on their back on a table. Another design principle of mobile power sources is a maximum power of 3 W, because of their heat generation. Prototypes of laptop fuel cells have been criticised for being noisy, bulky, and too heavy. When comparing the performance of batteries and fuel cells, one should be observant. Often the reported performance of a fuel cell does not take into account the whole system, only the cell itself without peripherals.

It is a hard task to replace secondary batteries as the power source for mobile phones. The size of these batteries is only 10-20 cm^3, compared to the physical size of 100-200 cm^3 of the corresponding state-of-the-art fuel cell powered chargers (Dumé, 2006). The first consumer products will most probably be wireless rechargers, as minimising the size of these is not as crucial as in a mobile phone. In the United States the military is offering a technology push for fuel cell development, and the Department of Defense (DoD) has been a major funder of different small fuel cell studies. The DoD's motivation is easy to understand. A modern soldier carries about 9 kg of batteries on average on a 5-day mission (Jane's, 2006). The fuel cells can offer a significant reduction in weight or permit longer mission times.

There are presently many competing technologies in the field of small fuel cells. Methanol-burning fuel cells have been in focus for several years. Even if methanol is a toxic and flammable liquid, it is considered more practical for small fuel cells than hydrogen. The direct methanol fuel cell (DMFC) is basically a modification of the PEMFC; its operation is explained in Figure 2.6 and Equations 2.3-2.4. The standard potential of the methanol|oxygen reaction is 1.21 V. The fuel is fed directly to the anode. This technology simplifies the design in principle, but there are also some penalties that must be paid, such as fuel crossover and lower efficiency. These will be discussed later on in Section 2.2.2. The DMFC is especially interesting for handheld devices, such as mobile phones and PDAs.

During the last few years the reformed methanol fuel cell (RMFC) has become more interesting, even in small appliances. Advances in micromechanics have made possible small enough components for the reformer unit. With reformation, greater efficiency can be achieved and the catalyst lifetime can be prolonged due to purer fuel feed. The reformed methanol fuel cell prototypes that have been demonstrated are intended only for laptops.

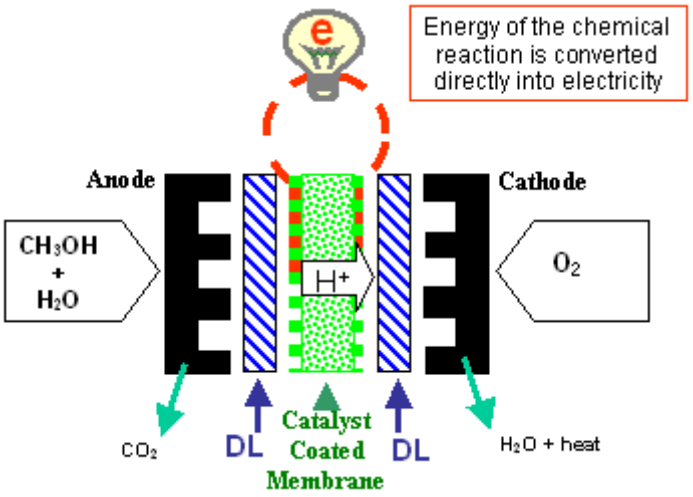


Figure 2.6. Operating principle of the DMFC. The anode and cathode are separated by a proton-conducting membrane. DL = gas diffusion layer. (Courtesy of MTI Micro Fuel Cells).

Anode: $CH_3OH + H_2O \rightarrow CO_2 + 6e^- + 6H^+$ (2.3)

Cathode: $3/2O_2 + 6e^- + 6H^+ \rightarrow 3\ H_2O$ (2.4)

$E^0 = 1.21$ V

The liquid electrolyte fuel cell (LEFC) is the result of research performed in Israel by the company Medis Technologies. The principal difference from the PEMFC and DMFC is the lack of a solid electrolyte. The alkaline liquid electrolyte and the fuel (an alcohol) are combined, together with additives, to make a proprietary mixture. The company claims their product is more environmentally friendly than basic alkaline batteries. Medis Technologies has a close relationship with the US military and their prototypes have been used in field testing in recent years.

The formic acid fuel cell and also the direct formic acid fuel cell (FAFC and DFAFC) are quite novel technologies. They are very similar to the DMFC, both in structure and chemistry. They have not been studied and developed as widely as methanol and hydrogen fuel cells, but show nearly the same performance as the DMFC. Rice et al. (2002) reported OCV of 0.72 V, current output 134 mA/cm^2 and power density of 48.8 mW/cm^2. Additionally, formic acid is not flammable like methanol. Another advantage over the DMFC is that the reaction can be catalysed with palladium-based catalyst material instead of platinum alloys, thus reducing the cost significantly (Larsen et al., 2005). A company called Tekion (USA) is developing mobile and portable prototypes of the DFAFC (Fuel Cell Review, 2005).

Table 2.3. List of different fuel cell technologies developed for small power sources.

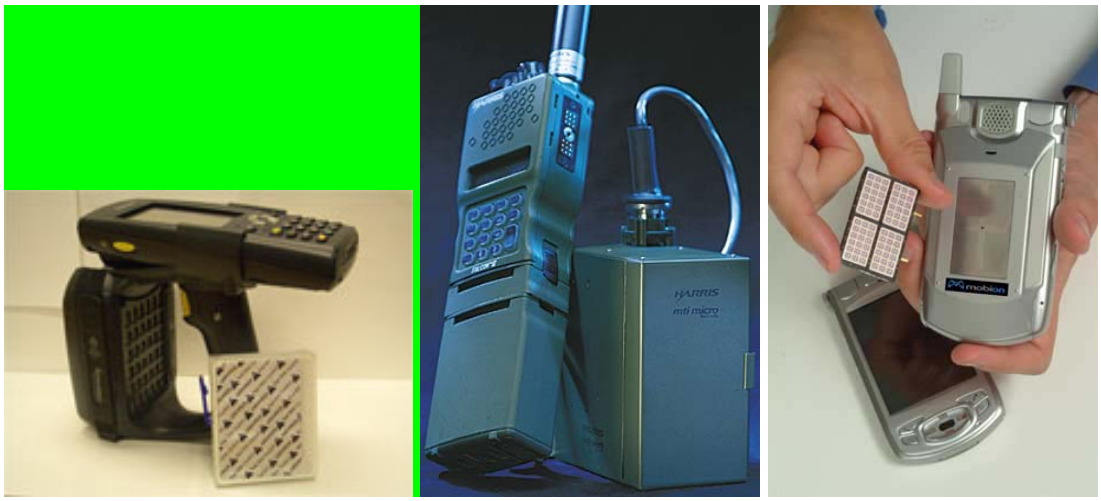
Acronym	Complete name	Fuel
PEMFC	Proton exchange membrane fuel cell	Hydrogen
DMFC	Direct methanol fuel cell	Methanol fed directly to the anode
RMFC	Reformed methanol fuel cell	Methanol reformed as hydrogen before being fed to a PEMFC
(D)FAFC	(Direct) Formic acid fuel cell	Formic acid
LEFC	Liquid electrolyte fuel cell	Proprietary fuel mixture, not disclosed
Bio FC	Biocatalytic fuel cell	Alcohols, carbohydrates, etc

The combination of fuel cell technology and biotechnology has created biological fuel cell technology. Biofuel cells, which utilise biological catalyst materials (i.e. enzymes or microorganisms), will be discussed in more detail in the next chapter.

The foremost companies in the area of mobile fuel cells are MTI Micro Fuel Cells (USA), Motorola (USA), Medis Technologies (Israel), Samsung (South Korea), and Toshiba (Japan). All of these companies are developing fuel cells for mobile phones and mobile phone chargers or for the military (Table 2.4). All except Medis use DMFC technology. MTI Micro Fuel Cells and Medis Technologies have prototypes of military tactical radiophones. One of the few developers of hydrogen micro fuel cells is Angstrom Power (USA). In Europe Smart Fuel Cell GmbH has developed portable applications (25-65 kilowatts) and recently published a

miniature device (Smart Fuel Cell). In Finland the company Hydrocell has taken a different approach to portable fuel cells; its fuel cell is based on AFC technology.

The prototypes of the MTI MicroFuel Cell are shown in Figure 2.7 below: an RFID reader with a spare fuel cartridge (a); a tactical radio with a fuel cell power source (b), and a fuel cell-powered conceptual mobile phone. MTI's proprietary technology, the Mobion™ fuel cell unit, has been used in the reader. The mass transport inside and air flow are passive and the fuel feed is 100% methanol. The size is reported to be less than 40 cm^3 (Baker et al., 2005).



a. *b.* *c.*

Figure 2.7. MTI Microfuel Cell's prototype of a DMFC for an RFID reader with a fuel cartridge (a); fuel cell-powered tactical radio (b), and a concept mobile phone with a Mobion™ DMFC (Courtesy of MTI Micro Fuel Cells).

In 2003 Samsung presented a prototype with a same surface area as that of a credit card (Chang, 2003). In 2005 Toshiba presented the world's smallest DMFC. Further development produced two prototype units: 100 mW (23 mm × 75 mm × 10 mm) and 300 mW (60 mm × 75 mm × 10 mm). The first one was able to power a flash-based player for 35 hours with 3.5 ml of methanol. The 300-mW unit produced a high enough voltage for a high-capacity MP3 player for 60 hours with 10 ml of fuel (Baker et al., 2005).

Table 2.4. Examples of mobile fuel cells prototypes.

Company	Application	Power output	Fuel/ oxidant	Catalyst (anode/ cathode)	Additional information
MTI Micro Fuel Cells (Gottesfeld, 2003)	Tactical radio	100 mW/cm^2 (170 mA/cm^2, 50.5 mW/cm^2)	100% methanol / Air	Pt-Rh/Pt	Orientation-independent, passive fuel feed, fuel usage 90%; 0.5-W proto for mobiles, 5-W (25-W peaks) military proto for tactical radios; cost estimate 3-5$/2-W system
Motorola (Bostaph et al., 2002)	Mobile phone recharger	100 mW/cm^2 1 W at 10 V (net output)	100% MeOH/ Air	Pt-Ru black/ Pt black	Active pumps, operation time > 1000 h, fuel usage 20%
Samsung (Chang, 2003)		32-50 mW/cm^2	2 M MeOH/ Air	PtRuRhOs, PtRuNiOs, PtRuRhNi / PtFe, PtCu, CuPd	2-W proto with 25 ml methanol, 2.6 h at 50 mA standby current, proprietary membrane
Toshiba (Baker et al., 2005)	MP3 player	100 mW	MeOH 100%/ air	na	Size: W22 x L56 x H4.5 mm Total, Weight: 8.5 g including 2 ml of methanol fuel, Fuel Tank Size: 2 ml, One fuelling runs MP3 player for 20 h

2.2.2 Technological considerations

Technological challenges in small fuel cells include the crossover of the fuel, poisoning of the catalyst, and water management. Also, in the case of DMFC, the contradiction between the low methanol tolerance of the platinum catalyst and the methanol concentration of the fuel has to be arranged in a feasible manner. To utilise low-concentration fuel would invalidate the high energy content of the fuel cell. Additionally, an important design principle for this type of fuel cell is to minimise the energy consumption of peripheral devices such as pumps and fans. Several techniques for passive fuel feed for DMFC have been suggested (Chang, 2003; McNamee and Acker, 2003; Guo and Cao, 2004; Ren et al., 2004). A passive reconstruction of the cathode is also challenging; changes in relative humidity have been observed to have a remarkable effect on the performance in mobile fuel cells with passive cathode (Stanley et al., 2005). Other issues are orientation independence and the ruggerised design of the power source.

The DMFC is considered the most suitable fuel cell type for mobile applications. Methanol is considered a more feasible fuel than hydrogen because hydrogen is a flammable and explosive gas and is typically pressurised, while methanol or another alcohol is easier to handle. A disadvantage is that it is toxic to humans (lethal dose 0.3-1 g/kg). If released into the environment, methanol is metabolised by microorganisms in soil (IPCS, 1997). Another more technological disadvantage is the crossover and incomplete oxidation, whose byproducts (such as carbon monoxide or formaldehyde) can poison the transition metal catalysts easily. The lack of reforming reduces the structural complexity of the device, which is beneficial considering miniaturisation, but the lifetime of the catalyst may be shorter than with a reformer as a result of the byproducts.

A well-known problem in DMFCs is the crossover of methanol to the cathode through the Nafion membrane. Methanol reacts with the cathode catalyst, thus harming the performance (short-circuiting). However, most groups still use Nafion. The properties of Nafion are far better than those of corresponding products. New membrane materials that leak less methanol, operate at low temperatures, and still have high proton conductivity have been under study. A serious competitor for Nafion is the hydrocarbon membrane developed at PolyFuel; this type of membrane is less expensive and makes possible thinner fuel cell structures. It passed 5000 hour durability tests during 2005 (Nano Battery, 2005). Chitosan membranes have also been suggested as replacements for Nafion; the material is low-cost but its thermal stability and proton conductivity need further development (Mukoma et al., 2004).

One limitation in the development of DMFCs has been the low methanol concentration the platinum catalyst tolerates (5-10%). The optimal methanol concentration for a DMFC is in the range of 0.5-2.0 M (1.6-6.4 %) (Guo and Cao, 2004). It is clear that the fuel used in a practical mobile device has to be more concentrated, preferably pure methanol. If the fuel cartridge contains only a dilute solution, the advantage over secondary batteries of (potentially) longer operation is lost. Water is needed to dilute the fuel; it can be fed to the anode or stored in the anode. A third possibility is to utilise water produced in the cathodic reaction (Figure 2.6 above). The consumption of water and methanol is theoretically 1:1 (Guo and Cao, 2004). Research groups and companies have proposed both active and passive fuel feed and dilution systems. The active systems consist of

micromechanical pumps, sensors, and a control unit and obviously consume energy produced in the fuel cell unit. The passive systems utilise capillary and diffusion forces to transport fluids inside the device.

Motorola has demonstrated a multi-layer ceramic technology for processing and delivering fuel and air to the fuel cell membrane electrode assembly (MEA). This fuel delivery system can be built into a miniature fuel cell. The MEA is constructed in such a way that four MEAs on one plate form the basic component with which the stack is built. They have patented a recovery and recirculation method for water. The prototype of a 1 W mobile phone charger generates 100 mW/cm^2 and uses pure methanol. However, the system utilises a micromechanical pump, which consumes about 50% of the energy produced by the fuel cell (Bostaph et al., 2002).

Clearly, a passive pumping system would be a better solution for a small, perhaps miniature, device. The small volume of the device already limits the amount of energy it can generate. Additionally, the amount of fuel that can be carried at a time is limited by the small size. Therefore, the amount of energy used by the system itself should be as little as possible, ideally negligible. Ren et al. (2004) have invented a passive fluid management component for a DMFC. The component allows pure methanol to be introduced into the anode. Its operation is based on specific pore sizes and pore spacing. The structure controls the feed of anode reactants (i.e. methanol and water from the cathode) to the anode. This component can also be formed in such a way that it extracts CO_2 from the anode effluent. Its inventors are connected with the MTI Microfuel Cell, which has a 5 W prototype for military tactical radio and a 0.5 W prototype for mobile phones. The power output is 100 mW/cm^2. Additionally, McNamee and Acker (2003) have developed a passive liquid feed for a DMFC system. The driving force of the pump is CO_2 formed in the anodic reaction. Furthermore, the pump is self-regulating because the amount of CO_2 produced is proportional to the power generated. A planar fuel cell for mobile phones has been developed in the Samsung Advanced Institute (Chang, 2003). The 2 W prototype is credit card-sized, albeit somewhat thicker, and produces 32-50 mW/cm^2.

To overcome the limitation of low methanol concentration in the fuel feed and to replace energy-consuming and complex micromechanical fuel feed systems, Guo and Cao (2004) presented another passive fuel feed system. The method was based on the wicking properties of a certain material. The wick material was porous and consists of ceramic, glass fibre, carbon fibre, polymers, and cotton. They tested their concept with an air-breathing DMFC. The methanol concentration in the anode chamber was maintained at the preferred concentration range for nearly 300 hours, during which the power output was stable.

At present some DMFC developers have returned to the reformation of methanol. Advances in micromechanics have paved the way to small enough reformers (Holladay et al., 2002; Pattekar and Kothare, 2004). Reformation increases the lifetime of the catalyst and the efficiency of the device.

Fuel cells have a constant power output. The power demand of most applications is typically uneven, with current peaks many times higher than in stand-by condition. If the fuel cell is designed to take care of the peaks, the advantage of higher energy density compared to secondary batteries is lost. To increase power output requires

more electrode area, that is, more unit cells and a larger volume. A feasible solution is to integrate a small secondary battery or a capacitor with a fuel cell. The battery shaves the power peaks as the fuel cell provides for the basic energy demand.

2.2.3 Commercialisation of small fuel cells

The buzz around small fuel cells has been going on for about a decade. So far, the only company known to be offering small customer fuel cells is the German Smart Fuel Cell. Every year the commercialisation seems to be pushed a couple of years further, as can be seen from Table 2.5, which lists the commercialisation plans of several companies in the year 2004.

During the last few years (2005-2006) the technological push seems to have been changing to market pull. The end-users – the military, battery manufacturers, and electronics companies – seem to have greater confidence than previously. Figure 2.8 below describes the development stage of small fuel cells in 2003, compared to secondary batteries. The expectation of the maximum energy density of a Li-ion battery is around 0.5 Wh/cm^3 (Gottesfeld, 2004). The promise shown by fuel cells is clearly seen in the graph (Fig. 2.8) assuming that the technological drawbacks are resolved.

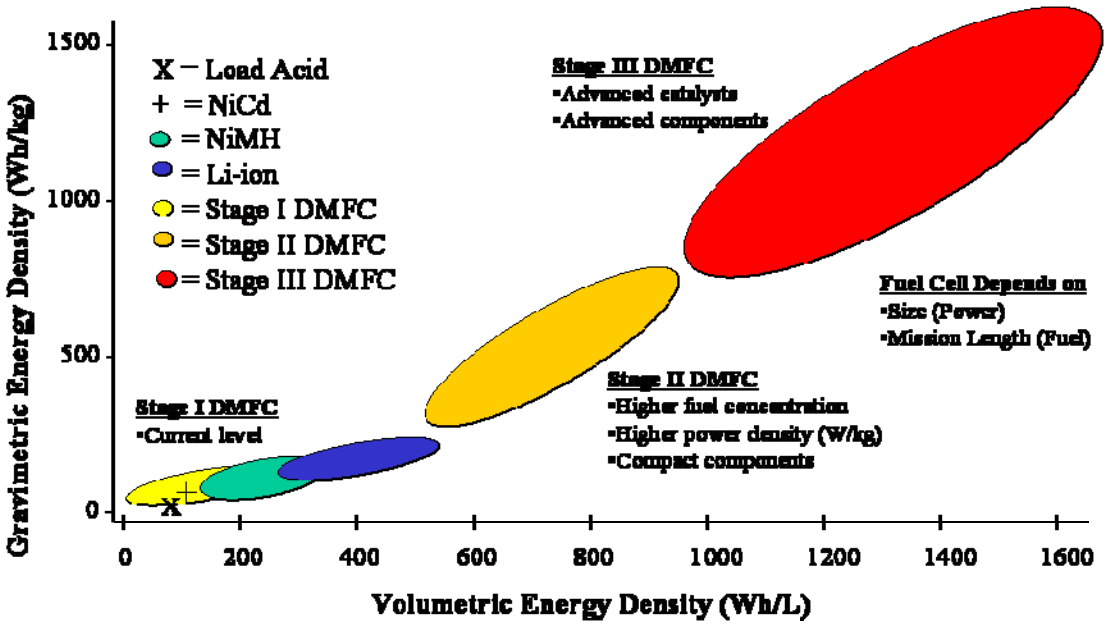


Figure 2.8. Status of fuel cell development and future potential of gravimetric and volumetric energy density compared with battery technology (adapted from Kelty, 2003).

Table 2.5 below illustrates two aspects of the commercialisation of mobile fuel cells: the optimistic estimations of commercialisation a few years ago and the expected first consumer product, which is the replacement of a laptop battery. In a recent survey on portable markets (Baker et al., 2005), the prognosis is similar. The electronics manufacturers involved in fuel cells aim at integrated battery

replacement, but among the developers they are few in number. The majority plan to progress from battery chargers to miniaturised integrated power sources.

Table 2.5. Commercialisation plans for mobile-use fuel cells (adapted from Kazama, 2004).

	2002	2003	2004	2005	2006	2007	Substitute for sec. batteries	Cartridges, aux. power sources
Casio Computer						↔	Notebook PCs	
Hitachi			↔				Notebook PCs	
Medis Technologies		↔						Cartridges
Motorola			↔	↔	↔			Mobile phones
MTI Micro-Fuel Cells			↔				Business-use terminals	
Neah Power Systems			↔	↔				0-5 to 40 W
NEC			↔	↔			Notebook PCs Mobile phones	
Polyfuel				↔				Mobile phones
Samsung Group			↔	↔			2 to 40 W	
Smart Fuel Cell	↔							Portable types, notebook PCs
Toshiba			↔	↔			Notebook PCs Mobile phones	

When considering mobile and portable fuel cells and their commercialisation, consumer attitudes and the lack of infrastructure, together with the remaining technological challenges, are very important factors. Even with a completely mature technology, effort is required to change people's minds about what is convenient and what is not. Charging batteries is considered easy, practical, safe, and fairly fast. It is easy to find sockets in public places in western countries. Refuelling with a fuel ampoule or cartridge is undeniably faster but one has to carry a spare cartridge. If the standardisation of fuel composition and cartridge types is not successful, one might have to carry several spare ampoules when travelling abroad, instead of an 110V/220V adapter; some chargers even have a built-in adapter. Amusingly, electric cars are in a similar situation, but the argument is the opposite: it is so fast to fuel a car and it takes hours to charge a battery.

The creation of global standards, legislation, and a fuel distribution infrastructure is a challenging task. At the moment methanol is listed as a dangerous chemical in most countries and it is not possible to carry it in aircraft cabins. The fuel cell industry has worked actively to advance the standardisation and legislation process. Smart Fuel Cells GmbH (DE) received approval for the air transport of their proprietary fuel cartridges as cargo in 2004. The Dangerous Goods Panel of the International Civil Aviation Organisation (ICAO DGP) is allowing the transport of methanol cartridges in aircraft cabins as of January 1, 2007 (Fuel Cell Today, 2005). The action covers micro fuel cells to be carried in the cabin and not as cargo, together with two spare fuel cartridges per passenger. The fuel cell types which were approved are: direct methanol, reformed methanol fuel cells, and those powered by formic acid and butane. The utilisation of hydrogen in metal hydrides and borohydride compounds was not included in the decision. The decision was submitted to the ICAO general meeting this year. Additionally, fuel cell power sources now have a design and performance specification (IEC Publicly Available Specification 62282-6-1) approved by the International Electrotechnical Commission (IEC); each device taken into an aircraft has to have an IEC certification. To get the final approval would have an important effect on the commercialisation of mobile fuel cells. Another challenge is that most fuel cell companies have proprietary cartridge designs and fuel compositions. To get

consumers, not only gadget fans, to approve the new technology, the fuel ampoules should be standardised in a similar way as batteries are today (A, AA, etc).

According to one expert opinion, early adopters of small fuel cells may be found in countries such as India, where electricity is not as inexpensive as in the western world and either wall sockets are not easy to find in public places or it is prohibited to use them. An example of the present commercialisation phase of mobile fuel cells is the contract between Samsung and MTI Microfuel Cells to introduce the Mobion technology in Samsung's new prototypes of mobile phones and accessories (Kotilainen, 2006). Samsung is relying on a market forecast according to which the fuel cell market would amount to about 80 million units in 2012.

2.3. Biological fuel cells

2.3.1. Introduction

The operation of the fuel cells described in Section 2.1-2.2 is based on metallic catalysts. The following section explains biological fuel cells and their technology and possible applications. If one compares the structure of PEMFCs and DMFCs to that of biofuel cells, one can find many similarities. Like all fuel cells, a biofuel cell has an anode and a cathode, which are separated by a proton-permeable layer. Additionally, the potential difference between the two electrodes must be large enough, as in conventional fuel cells.

The principal difference between the previously described fuel cells and biological fuel cells is the nature of the catalyst. An elementary definition of a biological fuel cell (later: biofuel cell) is that it is a device which transforms the chemical energy of a substrate, a fuel, directly into electricity by means of the catalytic power of biological matter. Palmore and Whitesides (1994) put it in the following way: "a biological fuel cell is a device which is based on the enzymatic reactions in either living microorganisms or of isolated proteins, the enzymes".

The principle of biofuel cells was discovered in the early 20th century by an English botanist, M. C. Potter (Potter 1912). Space exploration has had a strong influence on the study of biofuel cells. In the 1960s energy generation from waste and the urine or faeces of astronauts was under study. One of the cornerstones of the study of biofuel cells in the 1960s was the demonstration of electron transfer in a flavoprotein enzyme system by Yahiro (1964). In the late 1980s and early 1990s, interest in the study of biofuel cells started to awaken again, Bennetto being one of the most active microbial fuel cell promoters. In the field of enzymatic fuel cells Wingard, Palmore, Katz, Ikeda, and Kano have often been cited. In this thesis microbial fuel cells are only briefly explained. The principle of microbial fuel cells has been explained by Bennetto (1984) and Allen and Bennetto (1993), and more recently by Logan et al. (2006). An extensive review of the subject can be found in Bullen et al. (2006).

One benefit of biocatalysts is that they function in milder conditions, mostly in physiological conditions, and therefore inexpensive materials can be utilised for the fuel cell components. Another advantage is the broader choice of fuels. The utilisable fuels include carbohydrates (sugars), alcohols, waste water, and organic waste, to mention but a few. A third reason is the potentially less expensive catalysts, which are easy to mass-produce (e.g., production of the enzymatic additives in detergents). Having said that, it should be noted that platinum is not the major cost factor in metal catalyst fuel cells. The membrane is the biggest

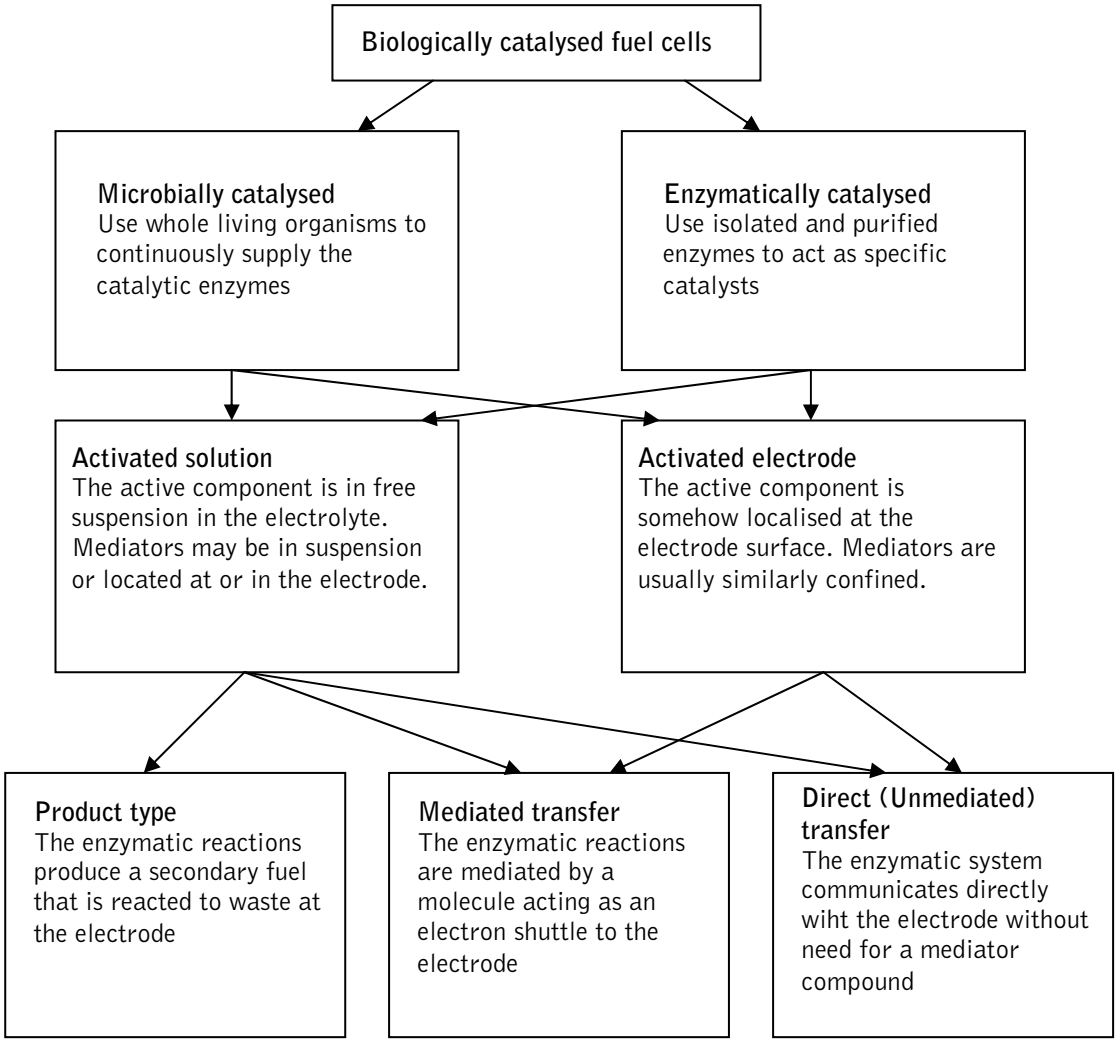


Figure 2.9. Classification of different types of biological fuel cells (adapted from Bullen et al., 2006).

individual cost. Additionally, a biodegradable power source could be created with enzymatic catalysts.

The disadvantages of biofuel cells are their lower power density and the shorter lifetime of the biocatalyst (both storage and operating lifetime). The highest reported power density of 5 mW/cm^2 was disclosed at a conference by the company Akermin (Akers, 2005). However, the test system and experiment conditions were not explicitly explained. The same group has published a power density of 1.6-2 mW/cm^2 in scientific forums (Akers et al., 2005).

Biofuel cells are categorised into two groups according to how the process is organised: direct and indirect. Indirect biofuel cells are those where the biological catalysis occurs in a separate reactor or chamber. The biological part of the process functions in the same way as a reformer. Biofuel cells that use living microorganisms as a catalyst are typically indirect. The microbial fuel cell set-up developed at the Automation Technology Laboratory is shown in Figure 2.10. The substrate is metabolised separately in the biofilm sponge. The electrons are carried from the reaction site to the fuel cell electrode by a mediator. A mediator is a chemical substance which is an electrochemically active and usually small-sized molecule able to penetrate cell walls. The mediator has a strong electron affinity

and is able to capture an electron from the reaction site and carry it to the electrode. In a direct biofuel cell the catalysis reaction occurs at the electrode chamber. The biocatalyst can be fixed or immobilised on the electrode (activated electrode) or be free in suspension in the electrolyte (activated solution) (Fig. 2.9). The direct biofuel cells are typically enzyme-catalysed biofuel cells.

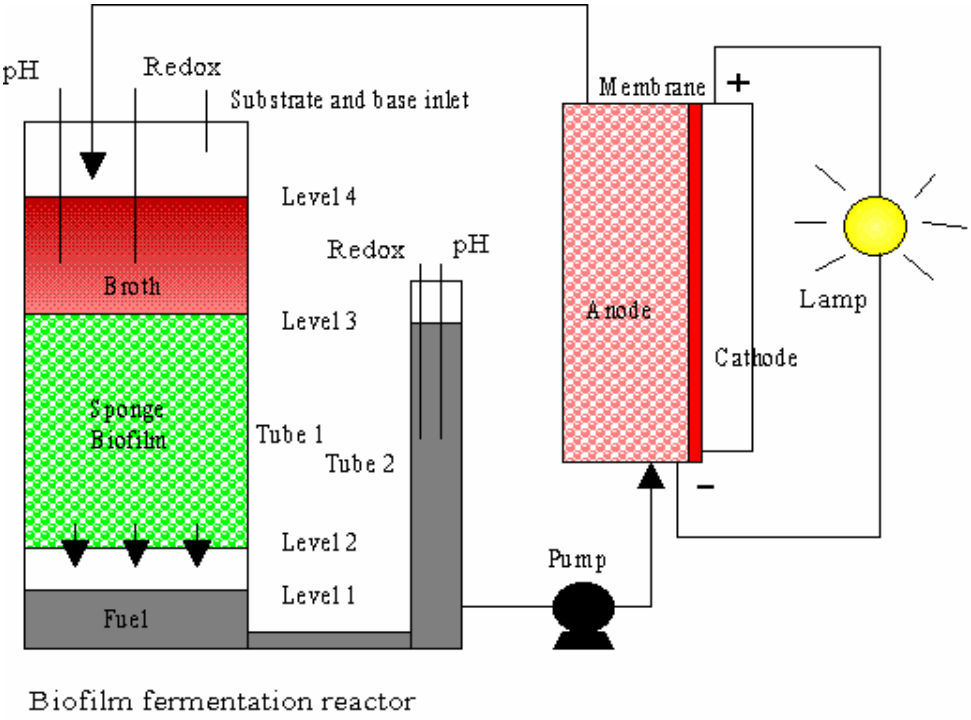


Figure 2.10. Set-up of the microbial fuel cell developed at TKK (Zhang, 1995). The fuel is reformed by the microorganisms in the bioreactor (on the left) and the charge is carried by the mediator to the separate fuel cell unit (on the right).

There are three different methods of electron transfer: the product type, mediated and unmediated electron transfer (Fig. 2.9). In the first one a secondary fuel is produced and reacted at the electrode. The mediated electron transfer is based on an electrochemically active mediator molecule as described above. Unmediated electron transfer between the enzyme and electrode surface is direct and does not require an additional step as in the mediated electron transfer.

The application areas are as the power source of electronics such as DMFCs, biodegradable disposable batteries (biobatteries), and the generation of energy from the sludge in waste water treatment plants or other organic waste treatment. The ultimate application would be self-fuelling or self-feeding robots.

2.3.2 Principle of biocatalytic power generation

Bioelectrocatalysis, the basis of biocatalytic power generation, describes the phenomenon associated with the acceleration of electrochemical reactions in the presence of biological catalysts, such as enzymes (Tarasevich, 1985). The biocatalyst can also be a microorganism as mentioned previously.

Energy-generative reactions occur naturally in living cells in animals and microorganisms. The natural metabolic reaction could be described as a "cold combustion" reaction. The living cell metabolises energy-rich substances, such as glucose, to provide it with energy at a physiological temperature (Eqs. 2.5 and 2.6). In the cell the extraction of energy contains the oxidation of fuel molecules via the citric acid cycle followed by oxidative phosphorylation. The electrons released in the reaction chain are carried by the intermediates, e.g. NADH. The terminal electron acceptor of the respiratory chain is oxygen.

$$C_6H_{12}O_6 + 6\ H_2O \rightarrow 6\ CO_2 + 24\ H^+ + 24\ e^- \quad (2.5)$$

$$6\ O_2 + 24\ H^+ + 24\ e^- \rightarrow 12\ H_2O \quad (2.6)$$

The metabolic reaction is based on enzymatic reactions inside the cells. The enzymes function in the same way as metal catalysts: they reduce the Gibbs free energy of activation. The enzymes are usually highly specific to their substrate, i.e. one enzyme expresses activity towards one substance. The enzyme recognises its substrate with a lock-and-key method. The steps of the enzymatic reaction are illustrated by Equation 2.7. First, a complex of enzyme and substrate, ES, is formed. In the second phase the enzyme changes the substrate molecule. Finally, the enzyme releases the product, P, without being changed in the reaction.

$$E + S \rightarrow ES \rightarrow E + P \quad (2.7)$$

The principle of a biofuel cell is depicted in Figure 2.11 below. In a fuel cell the electrons released in Reaction 2.5 would be captured at the anode electrode and led through an external circuit to the cathode electrode. The protons would pass through the membrane to the cathode, where these two would combine, together with oxygen or another terminal electron acceptor.

A paramount issue in any type of fuel cell is the efficiency of the electron transfer to the electrode. As mentioned earlier, the two principal methods of electron transfer utilised in biofuel cells are unmediated (or direct) and mediated transfer as mentioned earlier. Figure 2.12 depicts these two methods. The rate of electron transfer *in vitro*, between an enzyme and an electrode – an unmediated reaction – is naturally quite slow (Senda, 1990; Ikeda and Kano, 2001). The enzymes do not readily release electrons to an artificial electrode surface. Especially in the case of redox enzymes, a direct electron connection to the electrode is not possible, because of the enzyme's electrical insulation (Heller, 1990).

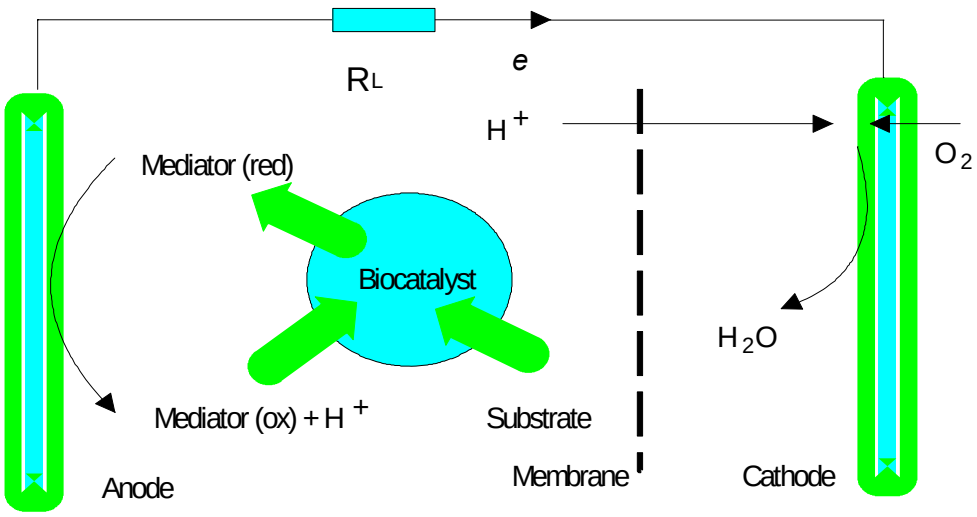
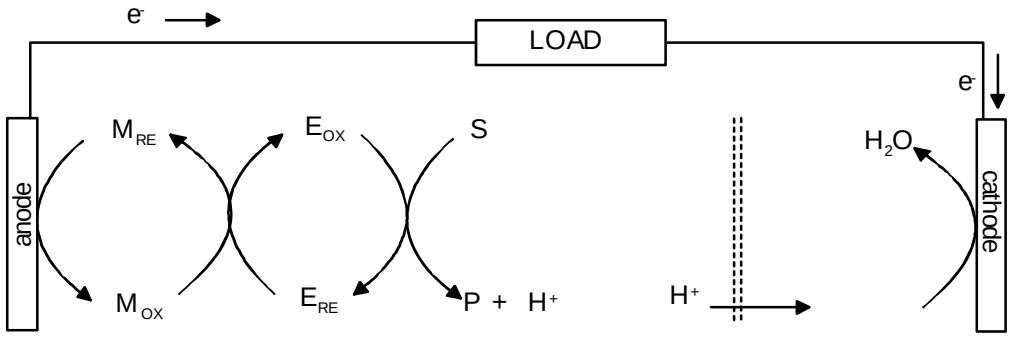
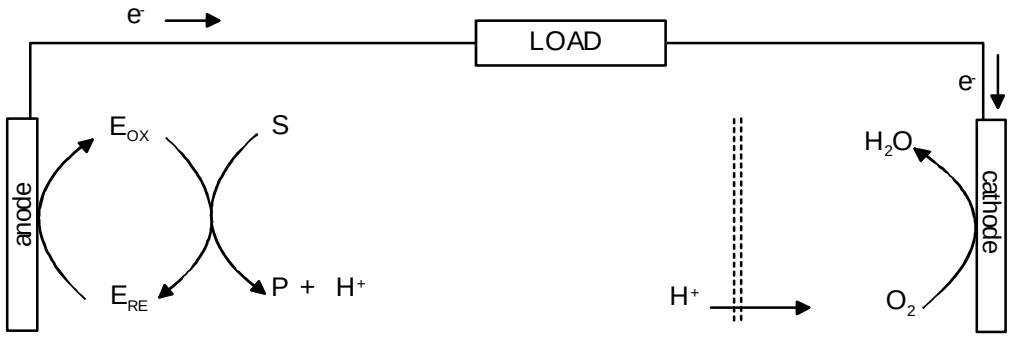


Figure 2.11. Operational principle of a biofuel cell.



a.



b.

Figure 2.12. Electron flow in mediated (a.) and unmediated (b.) bioelectrocatalysis. M_{RE} = reduced mediator, M_{OX} = oxidised mediator, E_{OX} = oxidised enzyme, E_{RE} = reduced enzyme, S = substrate, i.e., fuel, P = product, H^+ = proton, e^- = electron, H_2O = water, O_2 = oxygen

In mediated bioelectrocatalysis a mediator is utilised to transfer the electrons released in the enzymatic reaction from the vicinity of the enzyme to the electrode surface. A mediator is an electrochemically active substance which shuttles

between the activity centre of the enzyme and the surface of the electrode, changing its redox state. The enzymes are highly specific towards their substrates but the specificity to the mediator is not as limited (Senda, 1990). In theory, the mediator is not consumed in the reaction, but in practice a reduction in the number of active molecules has been observed (Zhang et al., 2006). Many of the suitable compounds are unstable or tend to oligomerise, to form chains of molecules, thus losing active electron carriers. According to Tarasevich (1985), a good mediator has to meet certain specifications: 1) there has to be fast interaction between the mediator and activity centre of the enzyme; 2) the oxidation-reduction potential of the mediator has to be near the enzymatic reaction, and 3) the mediator should be subject to electrochemical oxidation (or reduction) on the electrode under conditions close to reversible ones. Mediators that have been found utilisable are methylviologen (MV, E^0 = 0,011 V), tetramethylphenylenediamine (TMPD, E^0 = 0,26 V), and phenazine methosulphate (PMS, E^0 = 0,54 V). In a mediated reaction the potential of the mediator affects the overall potential of the biofuel cell. Thus it is favourable to select a mediator that has a potential close to that of the enzyme.

2.3.3 Enzymes as biological catalyst materials

Enzymes have many attractive features: high specificity, efficiency, moderate operating temperatures, physiological pH (in the general case, exceptions exist). Additionally, they can oxidise a greater variety of fuels than inorganic catalysts. Furthermore, they are renewable and inexpensive to produce. With genetic engineering, the enzyme can be modified to increase the efficiency or to widen the substrate specificity to a larger group of fuels.

The enzymes usually catalyse one step of the metabolic pathway. For example, the enzymatic oxidation of methanol to carbon dioxide requires 2-3 enzymes (see Equation 2.8). When considering the realisation of $MeOH|O_2$ fuel cells with biocatalysts, the right combination of enzymes is able to oxidise methanol to carbon dioxide at low overpotentials without producing carbon monoxide (Palmore et al., 1998). Successful combinations of enzymes in biofuel cell anodes have been published by e.g. Palmore et al. (1998) and Akers et al. (2005). Tables 2.6 and 2.7 list some of the most common enzymes in biofuel cell studies. It should be stated that the choice of anodic fuels and also of catalysts is more versatile than that of the enzymatic cathode, even though only a few are presented here. The oxidoreductase enzymes that can serve in biofuel cells (and also in biosensor electrodes) contain a specific functional group, a cofactor, for the electron transfer reaction. In some enzymes the cofactor is soluble (NAD^+/NADH). The soluble cofactor has to be added to the reaction solution; in this case the electron transfer is based on diffusion. The enzymes which are more practical in biofuel cell or biosensor electrodes have a more tightly bound prosthetic group or cofactor (PQQ, FAD, hemi, Cu^{2+}). Different alkanes or organic acids (lactate, malic acid) can serve as fuel.

Whereas for the cathode laccase and bilirubin oxidase are practically the only choices. In many biofuel cell studies the cathode reaction, reduction of oxygen, is performed by an inorganic catalyst, platinum, or silver. Until recently the study of the enzymatic anode was more popular than the enzymatic reduction of oxygen.

Active groups in the development of the enzymatic cathode include those of Heller (references under Mano et al. and Soukrahev et al.) and Palmore (Palmore and Kim, 1999). The typical cathodic enzymes are laccases, bilirubinoxidases, and peroxidases. They all possess a bound cofactor or prosthetic group and have a relatively high reduction potential. The first two, laccases and bilirubin oxidases, reduce oxygen and the peroxidases reduce peroxide to water.

$$\text{Methanol} \xrightarrow{MDH} \text{formaldehyde} \xrightarrow{MDH\ /\ AldDH} \text{formic acid} \xrightarrow{FDH} CO_2 \qquad (2.8)$$

Table 2.6. Feasible enzymes for biofuel cell anode.

Enzyme	Acronym	Substrate	pH	Reaction[1]
Alcohol dehydrogenase (NAD)	ADH	Ethanol	7	ethanol → acetaldehyde + 2 e^- + 2 H^+
Methanol dehydrogenase (PQQ)	MDH	Methanol	9.5	methanol → formaldehyde + 2 e^- + 2 H^+
Glucose oxidase (NAD)	GOx	glucose	5	glucose → glucolactone + 2 e^- + 2 H^+
Aldehyde dehydrogenase (NAD)	AldDH	Acetaldehyde, formaldehyde	8	acetaldehyde → acetic acid + 2 e^- + 2 H^+
Formate dehydrogenase (PQQ)	FDH	Formic acid	7.5	formic acid → CO_2 + 2 e^- + 2 H^+

[1] In the presence of water

Table 2.7. Feasible enzymes for biofuel cell cathode.

Enzyme	Acronym	Substrate	pH	Reaction[1]
Laccase (Cu^{2+})	lac	oxygen	4.5	O_2 + 4 H^+ + 4e^- → 2 H_2O
Bilirubin oxidase (Cu^{2+})	BOD	oxygen	8	O_2 + 4 H^+ + 4e^- → 2 H_2O
Peroxidase (hemi)		Hydrogen peroxide	5	H_2O_2 + 2 H^+ + 2 e^- → 2 H_2O

[1] In the presence of water

The maximum current generation with enzymes can be calculated in the following manner. The international unit of enzyme activity (IU) is defined as the amount of substrate (in micromoles) catalysed per minute (1 IU = 1 µmol of substrate consumed per minute at a specific temperature and pH). The enzyme reaction is usually known; most reactions release one or two electrons per substrate molecule. The generated bioelectrocatalytic current (and also the rate of the enzymatic reaction) is the number of electrons released per second (Kano and Ikeda, 2000). For example, Equations 2.9-2.10 show that the current generated by one enzyme activity unit for one electron reaction would be 1.6 mA per activity unit.

1 IU releases 1 µmol of electrons per minute;
thus $n(e^-)$ = 1 µmol and t = 60 s

$$Q = nF = 1 \times 10^{-6}\ \text{mol} \times 96485\ \text{C/mol} = 9.65\ \text{C};\ F = 96485\ \text{C/mol} \qquad (2.9)$$

$$I = Q/t = 1.6\ \text{mA};\ t = 60\ \text{s} \qquad (2.10)$$

However, the maximum current output can usually be achieved only momentarily, if at all. In addition, many enzymes usually function at lower rates than the ones determined at optimal initial rate conditions.

2.3.4 Review on recent enzymatic fuel cell studies

The simplest biofuel cell design is based on a soluble enzyme and a mediator, where the enzyme and mediator are free in solution and unattached to any support or carrier material. Nevertheless, efficient electron transfer may be difficult or impossible to arrange in this case because of the relatively long distance between the electron transferring mediator and the electrode surface. Bringing the enzyme and the mediator into close contact with the electrode enhances the electron transfer rate substantially. In addition, immobilisation can stabilise the enzyme and prolongs its lifetime. Traditional immobilisation methods from biotechnology are also applicable in the construction of biofuel cell electrodes. Two efficient novel methods have been developed; one is so-called "wiring" (Heller, 1990; Mano et al., 2002; 2003a; 2003b; 2004) and in the other one the enzyme is entrapped in modified Nafion (Akers et al., 2005).

Wiring was first designed for biosensor use and refers to the electrical connection of biomolecules to redox polymers in order to achieve good transduction to the electrode (Katakis and Heller, 1997). The concept is illustrated in Figure 2.13 and in more detail in Figure 2.14 below. The concept consists of three parts: the biomolecule (enzyme) that is responsible for the catalytic reaction; the redox polymer (the wire) that is responsible for electron transfer, and the surface to which the redox polymer is fixed (electrode or optically active material). Heller (1990) proposed osmium containing redox hydrogels, which can be utilised with anodic and cathodic enzymes.

The presented technique makes possible membraneless biofuel cells and ultimately the realisation of an implantable glucose sensor (Mano et al., 2002, 2003a, and 2003b). The operation of an implantable glucose sensor has been verified in a grape (Mano et al., 2003a). Mano et al. (2003b) have also reached a high operating voltage, 0.78 V, with a power output of 1.2 µW corresponding to a power density of 268 µW/cm^2 (37 °C, pH 5) in a fully biological fuel cell. Further development of the redox polymer has resulted in a cathode material that is reported to have better oxygen-reducing capabilities than platinum and an operating voltage of 0.88 V and a power density of 350 µW/cm^2 (Soukrahev et al., 2004).

A MEA-like approach has been presented by a research group at the University of St. Louis and a spin-off company, Akermin Ltd. The aim of the study is to develop a device that could compete with DMFCs. Their approach is to modify Nafion so that enzymes could be immobilised in it (Moore et al., 2004; Akers et al., 2005). Nafion is too acidic in its normal state to be used as carrier material. However, Nafion treated with quaternary ammonium salt forms a neutral suspension which can be utilised to immobilise enzymes. The method protects and stabilises the enzymes. Thus it is possible to create an enzyme-modified MEA.

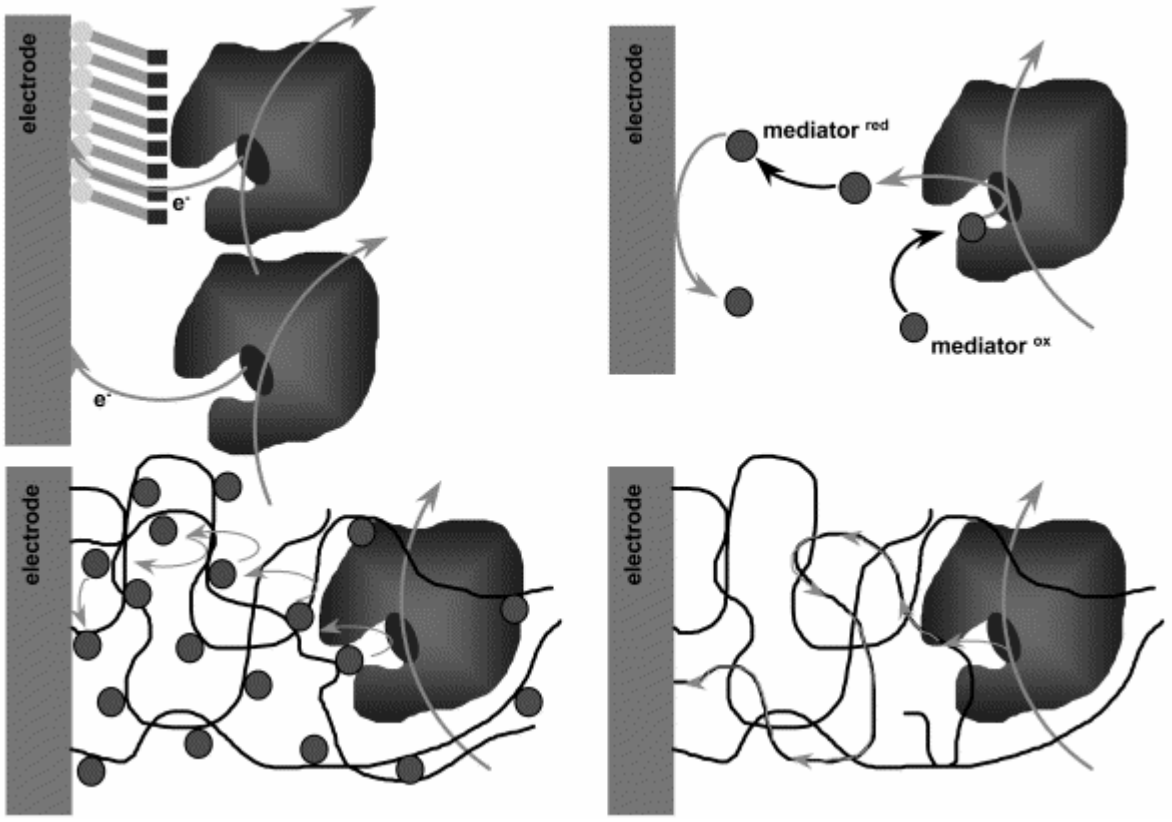
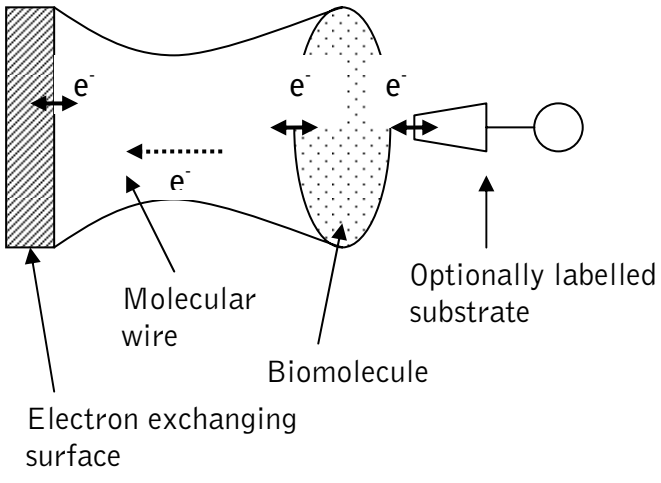
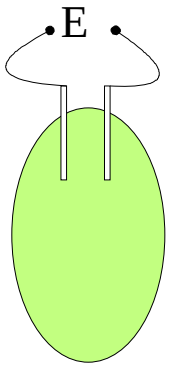


Figure 2.13. Methods of electron transfer from the enzyme's active centre to the electrode: direct transfer (above left); hopping (below left); mediated (above right), and wiring (below right). Reprinted from J. Biotechnol., 82 (4), Schuhmann, W., Amperometric enzyme biosensors based on optimised electron-transfer pathways and non-manual immobilisation procedures, 425-441, Copyright (2002), with permission from Elsevier.





a. *b.*

Figure 2.14. a. Enzyme wiring (adapted from Katakis and Heller, 1997); b. testing an implantable biofuel cell in a living plant (grape) (adapted from Mano et al., 2003b).

Table 2.8. Examples of biofuel cell studies found in the literature.

Reference	Catalyst A = anode C = cathode	Anode Fuel	mediator	pH	Anode material	Cathode fuel	Cathode material	Open circuit voltage	Current	Power density
Zhang & Halme, 1997	A: Flora of sea sediments[1] C: Ag	Glucose, fish	HNQ	8.8	Graphite particle	O_2	Oxygen diffusion Ag-gauze	0.9 V	25 mA	420 µW/cm^2
Yahiro et al., 1964	A: MDH C: Pt	Methanol, formaldehyde	PES, PMS	7.0	Pt gauze	O_2	Pt-gauze	0.3 V	3.7 mA at 10 Ω	19 µW/cm$^{2\ 2}$
Katz et al., 1999	A: Gox C: COx	Glucose	A:(PQQ)-FAD C: Cyt c	7.0	Au plate	O_2	Au-plate	130 / 160 mV	na	5 µW/ cm^2 at 0.9 kΩ
Palmore et al., 1998	A: FDH+AldDH +ADH & diaphorase C: Pt	methanol	NAD^+/NADH & benzylviologen	7.5	Graphite plate	O_2	Pt-gauze	0.8 V	na	0.67 mW/cm^2 at 0.49 V
Mano et al., 2002	A: GOx C: BOD	Glucose	Polymer I & II[3]	7.2	Carbon fibre	O_2	Carbon fibre	na	na	4.3 µW/mm^2 at 0.52 V
Mano et al., 2003a	A: GOx C: laccase	Glucose	Polymer I & II[3]	5	Carbon fibre	O_2	Carbon fibre	na	na	2.68 µW/mm^2 at 0.78 V
Mano et al., 2004	A: GOx C: BOD	Glucose	Polymer I & II[3]	7.2	Carbon fibre	O_2	Carbon fibre	na	na	4.8 µW/mm^2 at 0.6 V

Table 2.9. continue. Examples of biofuel cell studies found in the literature.

Reference	Catalyst A = anode C = cathode	Anode Fuel	mediator	pH	Anode material	Cathode fuel	Cathode material	Open circuit voltage	Current	Power density
Soukrahev et al., 2004	A: GOx C: laccase	Glucose	Polymer I & II[4]	5	Carbon fibre	O_2	Carbon fibre	na	na	350 µW/ cm^2 at 0.88 V
Akers et al., 2005	A: ADH + AldDH C: Pt	ethanol	NAD^+/NADH & methylene green	neutral	Carbon felt	O_2	Gas diffusion electode with Pt	0.61-0.82 V	Approx. 3 mA/cm^2 [5]	1.16-2.04 mW/ cm^2
Akers et al., 2005	Same as above	methanol	Same as above	neutral	Same as above	Same as above	Same as above	0.71 V	Approx. 3 mA/cm^2 [5]	1.55 mW/ cm^2
Zhang et al., 2006	A: MDH C: no catalyst	methanol	TMPD	9-10	Graphite foil	$KMnO_4$	Graphite foil	1.4 V	0.38 mA/cm^2 at 0.67 V	0.25 mW/cm^2 at 0.67 V

[1] The anodic catalyst was a natural mixture of bacteria and algae, which is found in the sediments of the Baltic Sea.
[2] Power density was calculated with given information and anodic area.
[3] Redox polymers based containing osmium for the electron transfer.
[4] Cross-linked Polymer I and II.
[5] The approximate value was read from the graph in the article referred to.
na = not available
Acronyms: ADH = alcohol dehydrogenase; AldDH = Aldehyde dehydrogenase; BOD = bilirubin oxidase; COx = cytochrome oxidase; Cyt c = cytochrome c; FAD = flavin adenine dinucleotide; FDH = formate dehydrogenase; GOx = Glucose oxidase; HNQ = 2-hydroxy-1.4-naphtoquinone; MDH = methanol dehydrogenase; NAD^+/NADH = nicotinamide adenine dinucleotide; PES = phenazine ethosulphate; PMS = phenazine methosulphate PQQ = pyrroloquinoline quinone

At first the group utilised NAD-dependent dehydrogenases but later started to utilise NAD-independent enzymes which do not require an external cofactor. In 2005 they claimed to have a biofuel cell with this set-up in operation with a run time of 356+ days and producing a power density of 5 mW/cm^2 (Akers, 2005). It was not clear whether the device had an enzyme-modified MEA or was a test bench set-up. Nor were the experiment conditions disclosed in a clear manner. The P/I curve shown in the same document showed a power maximum of approximately 1.15 mW/cm^2 at a current of 2 mA/cm^2. The biofuel cell ran on ethanol and had PQQ-alcohol dehydrogenase at the anode and bilirubin oxidase at the cathode for the oxygen reduction.

As previously described, most biofuel cells are like metal catalyst fuel cells and have separate anode and cathode compartments. Katz et al. (1999) and Mano et al. (2002) have proposed a non-compartmentalised glucose$\mid O_2$ biofuel cell, where the proton exchange membrane is excluded from the composition. The substrate specificity of the enzymes allows membraneless realisation if both the anodic and cathodic catalysts have pH optima close to each other. The membraneless structure is beneficial for the miniaturisation of the biofuel cell: fabrication of the membrane on a micro scale is demanding, as is sealing the device (Mano et al., 2002). The substrate specificity of enzymes was also exploited in the conceptual micromechanical biofuel cell proposed by Kjeang et al. (2006), in which the mixed fuel and oxidant flows were mixed.

Table 2.8 above lists selected biofuel cell studies, together with the results of the microbial fuel cell developed at the Automation Technology Laboratory.

2.3.5 Applications of biofuel cells

Potential applications of biofuel cells utilising enzyme catalysts are as power sources for consumer electronics and miniature robots or implantable power sources. A mobile power source could replace secondary batteries or it could be equivalent to primary batteries, but would be more environmentally friendly or even biodegradable. The concept of the biobattery has been presented by Wilkinson (2001) and Liberatore et al. (2002). An implantable power source, could, for example, run a blood sugar level monitoring and control system, deriving the energy it needed from the glucose and oxygen in a person's blood circulation. A similar medical approach is as the power source of a pace-maker, which could derive the energy they needed from the patient's blood circulation in the future. A third potential medical application could be in prosthetic valves.

However, real-life applications of biofuel cells to power electronics are hard to find. The principle of bioelectrocatalysis has already been applied in practice in sensor technology (biosensors). Plotkin et al. (1981) have demonstrated a methanol detector in which the biocatalyst was methanol dehydrogenase (from *Pseudomonas* AM1, currently classified as *Methylobacterium extorquens*) and PES the mediator. They developped an extremely sensitive detector, which was able to accurately detect methanol concentrations down to $5{\cdot}10^{-11}$ moles and straight chain primary alcohols (up to n-decanol) over the range 10^{-7} to $5{\cdot}10^{-10}$ moles.

There are niche markets such as the implanted blood sugar level monitor developed in cooperation with Heller and his co-workers and Therasense Co, presently Abbott Diabetes Care. The system, Freestyle Navigator™ was submitted for FDA approval in the USA in 2005. Bazhang et al. (2006) have also developed one.

If one considers the potential applications for microbial fuel cells, treatment of waste water or organic waste is the first area one thinks of. Integrated to an organic waste treatment process a microbial fuel cell could provide extra energy and probably somewhat decrease the amount of final output. In the 1960s this was also studied in the space exploration projects. Recently, the ESA (European Space Agency) funded a conceptual study of the same subject which studied how to derive power from faeces and other organic waste (Zhang et al., 2005).

A more futuristic application area is the robotics: a biofuel cell-powered robot would be able to forage for its fuel like cattle. Generally speaking a fuel cell-powered robot would have more independence of operation because of higher power density. The concept of a gastrobot, an eating robot, was postulated by Wilkinson in the late 1990s. Wilkinson and Campbell (1996) suggested several renewable energy sources for electricity generation for miniature robots: light, heat, fluid flow, and food-powered.

Wilkinson's group at the University of South Florida has realised a food-powered robot named the Gastrobot (Wilkinson, 2000a; 2000b; 2001). This fascinating bio-mimetic approach proposes the possibility of energy self-sufficiency in unstructured outdoor environments. The gastrobots are powered by a microbial fuel cell, which acts as an artificial stomach. The gastrobots could serve as lawn movers or as helpers patrolling in citrus groves, monitoring soil moisture levels and insect infestations (Wilkinson et al., 2001). They investigated different natural foodstuffs and came to the conclusion that the most practical choice of energy source is vegetation: grass, hay, or fruit (Wilkinson, 2000b; 2001; Wilkinson et al., 2001a). In one of the development lines honey and citrus fruits were studied as fuel. The juice of one orange (~100 ml), together with 1.5 tablespoons of honey, yields 37 Ah at 1.2 V, which corresponds to 8 fully charged Ni-Cd D-cell batteries.

Figure 2.15. The gastrobot (Chew-Chew) runs on sugar cubes. (Courtesy of Stuart Wilkinson)

Natural foodstuffs create some challenges when used in microbial fuel cells. Many of the microorganisms which are useful as biocatalysts are facultative enteric bacteria (e.g., *E. coli*, *Proteus vulgaris*) and have the optimal pH of around 7

(Wilkinson et al., 2001a). Most of the foodstuffs are more or less acid. A low pH can decrease the activity of the microorganism. Another specific problem is waste management, or how to get rid of the "undigested" parts of the food (Wilkinson, 2001). Another food-powered robot, the SlugBot, has been developed by Kelly (2003). This robot feed on insects. In the underwater world autonomous robots could utilise plankton as fuel.

As a practical power source the biofuel cell has to fill a credibility gap, a gap of performance. Many applications require a power density of 10-100 mW/cm^2 (Bullen et al., 2006), and the power density of biofuel cells is below that. Nevertheless, one has no reason to doubt that the technological issues cannot be solved in the future. For example, Heller's group has been able to increase the power density 80-fold in a couple of years (see Table 9 above: Mano et al., 2002; Soukrahev et al., 2004). Advances in biotechnology, materials science, and micromechanics will undoubtedly bring about novel solutions and methods.

Bullen et al. (2006) and Davis and Higson (2006) have recently published thorough reviews of biofuel cell study. Both groups concluded that biofuel cell developers still have some way to go. Niche markets, such as glucose sensors for diabetics, do exist, but generally there is a performance gap when compared to regular small fuel cells and, of course, an even larger gap compared to batteries. A review of earlier biofuel cell studies has been written by Palmore and Whitesides (1994).

Chapter 3

DESCRIPTION OF THE DMBFC AND THE EXPERIMENTS

3.1 Concept of the direct methanol biocatalytic fuel cell

The Direct Methanol Biocatalytic Fuel Cell (DMBFC) concept is based on an energy generation process utilising bioelectrochemical catalysis (Halme et al., 2002a; 2002b; Ranta et al., 2003). The system covers the realisation of the actual power source covers the complete fuel cell system, with a biological catalyst, fuel cell unit, fuel tank, and electronics, and is a stand-alone device (Fig. 3.1). As the name implies, the biofuel cell that has been developed has a direct fuel feed to the anode. The components, test set-up and experiments will be described in this chapter. The operational principle is described in Chapter 4.

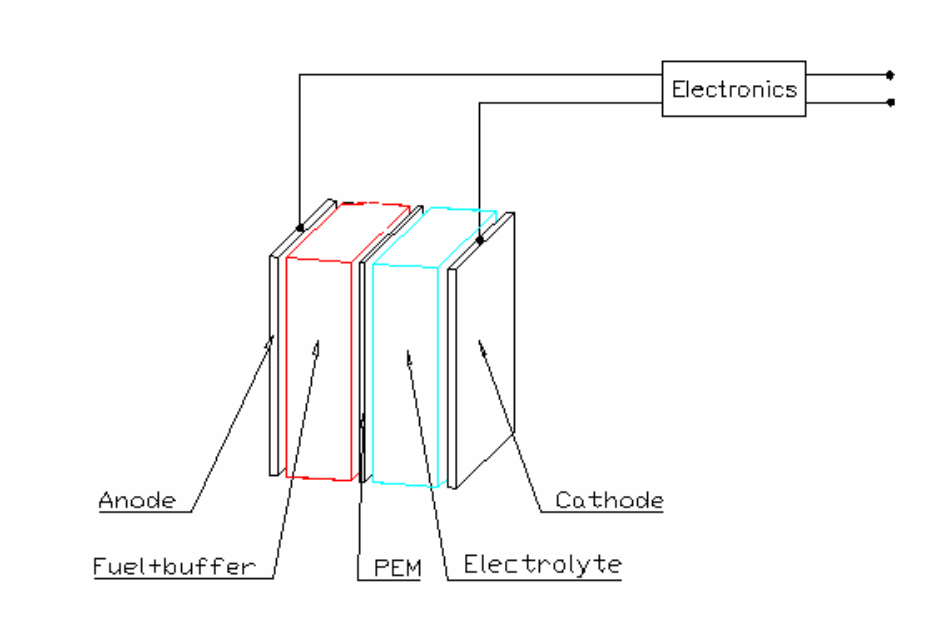


Figure 3.1. Concept of the DMBFC

3.2 Description of the DMBFC system

3.2.1 General aspects

Figure 3.1 illustrates the structure of the DMBFC. For the sake of simplicity, only one unit cell represents the fuel cell. The terminology used in this thesis may differ somewhat from that used in the conventional fuel cell community. Therefore, the terms describing the components of the DMBFC are explained in Table 3.1 below.

The operation of the anode is based on an enzymatic catalyst. The enzyme is applied to the electrode in two different ways: 1) in a unit cell the enzyme is fixed into a graphite paste with the mediator, and 2) in a stack construction it is mixed into the anodic electrolyte. A free enzyme in suspension was also used in earlier test unit cells. The anodic solution contains a buffer and a fuel, while in a stack DMBFC the mediator is also in the solution. Graphite foil or perforated steel plates are used as the current collector materials.

Table 3.1. Terminology

Component or compound	Description of the term
Fuel cell system	The fuel cell unit, electronics, peripherals, fuel tank, and oxidant tank if closed cathode type
Biofuel cell	A fuel cell with an enzymatic anode or cathode or both
Unit cell	Entity comprising an anode, a cathode, and a membrane
Double cell	Two unit cells sharing the fuel tank
Stack	N unit cells connected together (N > 2)
Anode	Negative electrode: active components on a support
Bioanode, biocathode	Operation based on a biological catalyst: enzyme or microorganism
Cathode	Positive electrode: active components on a support
Closed cathode	Terminal electron acceptor is stored inside the cathode chamber
Air cathode	Atmospheric oxygen as terminal electron acceptor
Current collector	Highly conductive material on top of electrode support
Electrode backing/support	Rigid material used to stabilise the structure
Electron conductor	A component of the paste: enhances conductivity (graphite powder)
Membrane	A selective membrane between the anode and cathode
Binder	Component of the paste: improves the processability of the paste
Fuel	Source of electrons
Mediator	An electrochemically active compound, which shuttles electrons from the activity centre of the enzyme to the electrode surface and is not consumed in the reaction
Oxidant	Terminal electron acceptor
Paste	A thick mixture spread on top of the current collector; consists of binder, electron conductor, and stabiliser, with or without the enzyme and mediator
Stabiliser	Component of the paste: a chemical which enhances the performance/lifetime of the enzyme and/or mediator

An electrical model of the DMBFC is illustrated in Figure 3.2. An interesting part of the model is the activation resistance R_A and the capacitance C_A related to it. In a metal catalyst fuel cell R_A is caused by the slowness of the reactions at the electrodes and C_A by the charge double layer phenomenon, an important factor in the dynamic behaviour of the fuel cells. It is a thin layer of charge at the interface between the electrode and electrolyte and acts similarly to a capacitor, in that it stores an electrical charge and energy. The activation resistance varies according to the operating point of the fuel cell.

In a biofuel cell the activation resistance is a product of the dynamics of the enzymatic reaction and the electron transfer rate to the anode (i.e., the activity of

the mediator). The C_A functions in the same way as in a metal catalyst fuel cell. The internal resistance R_{IN}, which is an important parameter in a biofuel cell process, is the sum of the activation resistance R_A and the ohmic resistance R_Ω.

$$U = RI = (R_L + R_A + R_\Omega)I \quad (3.1)$$

$$R_{IN} = R_A + R_\Omega \quad (3.2)$$

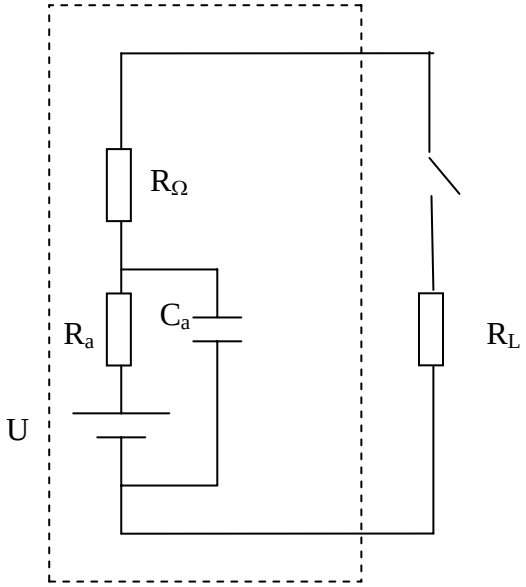


Figure 3.2. Electric model of the DMBFC. R_L is the loading resistance, R_Ω the ohmic resistance, R_A the activation resistance, and C_A the capacitance related to the reaction. U is the voltage.

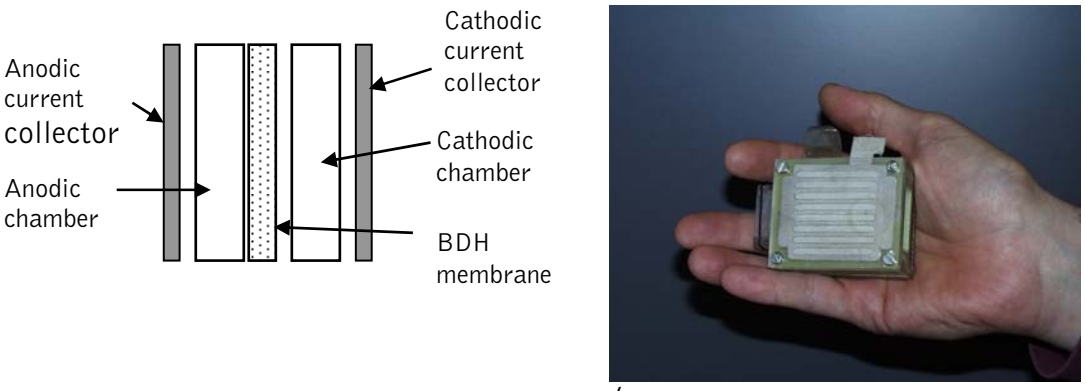
3.2.2 System description of the DMBFC unit cell

The structure of the unit cell is illustrated in Figure 3.3 and its properties are shown in Table 3.2 below. Two types of constructions were tested: an enzymatic anode together with 1) a passive oxygen diffusion cathode, or 2) a closed cathode having potassium permanganate as the terminal electron acceptor. It should be stressed here that both types of cathode were non-enzymatic.

The enzyme was fixed into the anodic paste. The composition of the paste was as follows: methanol dehydrogenase MDH (2 units), fine graphite particles, paraffin oil. The paste was spread onto the anodic current collector, which was graphite foil (thickness 0.25 mm). The thickness of the paste layer, i.e., the catalyst layer, was 0.5 mm. The free volume inside the anode chamber held the buffer solution, 0.2 M K_2HPO_4-NaOH pH 10, and the mediator, 5-8 mM TMPD. The methanol concentration was generally 20% or less, but concentrations up to 40% were tested. The two electrode chambers were separated with a cation exchange membrane (BDH no. 55165, dry thickeness 117 µm, wet thickness 118-120 µm).

As described above, two kinds of cathodes were tested: an oxygen diffusion cathode manufactured by Gaskatel GmbH (DE) and a potassium permanganate cathode,

i.e. a closed cathode. The air cathode was platinum-free. The reduction of oxygen was based on silver and nickel as the catalytic materials. The principle of a closed cathode is to include the oxidant in the system. In this case the chosen oxidant was potassium permanganate. The cathodic current collector was graphite foil. Silver wire was utilised as the external connection. Regular alligator clips were used for the electrical connections. The area of the electrodes was 16 cm^2. Plastics such as acryl, nylon, and polyoxymethylene (POM) were utilised as casing materials. The thickness of the outer materials was not optimised.



a. *b.*

Figure 3.3. The DMBFC unit cell utilised in experiments. a. Schema. b. The unit cell in the photo is equipped with the air cathode; the vertical cuts on the cover allow oxygen flow. In the case of the closed cathode the cover was solid plastic.

Table 3.2. DMBFC unit cell properties.

ANODE		CATHODE	Oxygen diffusion cathode	Closed cathode
Buffer	0.2M K_2HPO_4-NaOH	Buffer	0.2M K_2HPO_4-NaH_2PO_4	0.4 M $KMnO_4$ in H_2SO_4
Mediator concentration	TMPD 5-8 mM	pH	6	2
Enzyme concentration	MDH 2 units	Current collector	Nickel net & graphite	Graphite foil
pH	10	Terminal electron acceptor	Atmospheric oxygen	K_2MnO_4
Paste	Graphite: paraffin oil (2:1)	Surface area	16 cm^2	16 cm^2
Current collector	Graphite foil	Volume	15-20 ml	15-20 ml
Fuel	MeOH max 20%			
Surface area	16 cm^2			
Volume	15-20 ml			

3.2.3 System description of the DMBFC stack

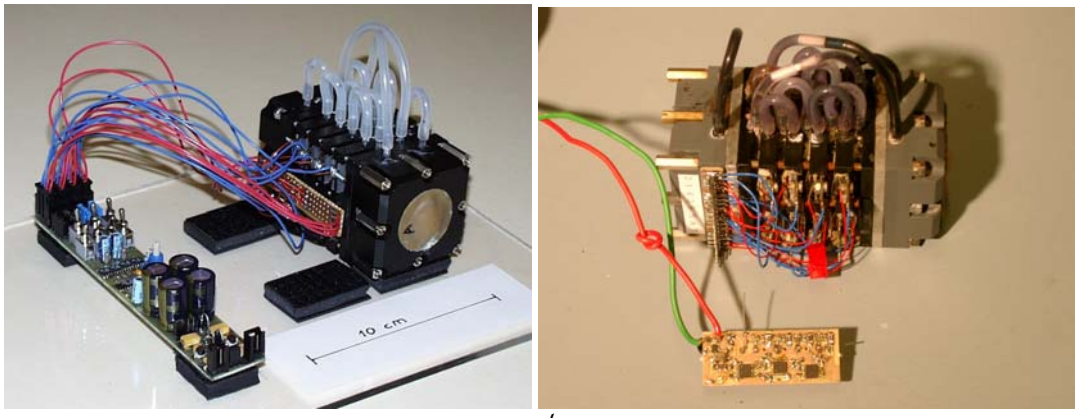
The eight unit cell stack was made of plastic (POM and nylon). The liquid flow between each unit cell was realised by connecting individual chambers together with plastic tubes. The BDH membrane separated the anode and cathode, as before. Perforated metal plates of stainless steel served as current collectors at the anode and graphite foil at the cathode. The structure was sealed with parafilm foil. The internal fluid flow was made possible with a manual membrane pump at each end of the stack (Figures 3.4-3.5 and Table 3.3).

Table 3.3. Properties of the eight-cell DMBFC stack.

ANODE		CATHODE	
Buffer	0.2 M K_2HPO_4-NaOH		0.2 M $KMnO_4$ – 25% H_2SO_4
Mediator concentration	8 mM TMPD	pH	2
Enzyme concentration	MDH 24 units	Current collector	Graphite foil
pH	10	Volume	21 ml
initiator	21.6 mM NH_4Cl		
Current collector	Steel plate		
Surface area	58 cm^2		
volume	24 ml		

The anodic solution was deoxygenated, at first with nitrogen gas and finally in a deoxygenator chamber overnight. The stack was filled with nitrogen gas and also put inside the deoxygenator to wait to be filled up with the solutions. The insertion of the anodic solution was done in such a way that the liquid would fill the chamber completely, leaving no gas bubbles or only a few. The enzyme was added halfway through the filling of the anodic volume. The volume of enzyme solution was equivalent to 24 units.

The cathodic solution was recycled with a peristaltic pump after it was clear that a manual pump was inadequate.



a. *b.*

Figure 3.4. a. DMBFC stack with the first-generation electronic circuit showing the manual membrane pump (the lighter circular part on the end plate) and b. the stack with the next-generation electronics (Salcomp Oy).

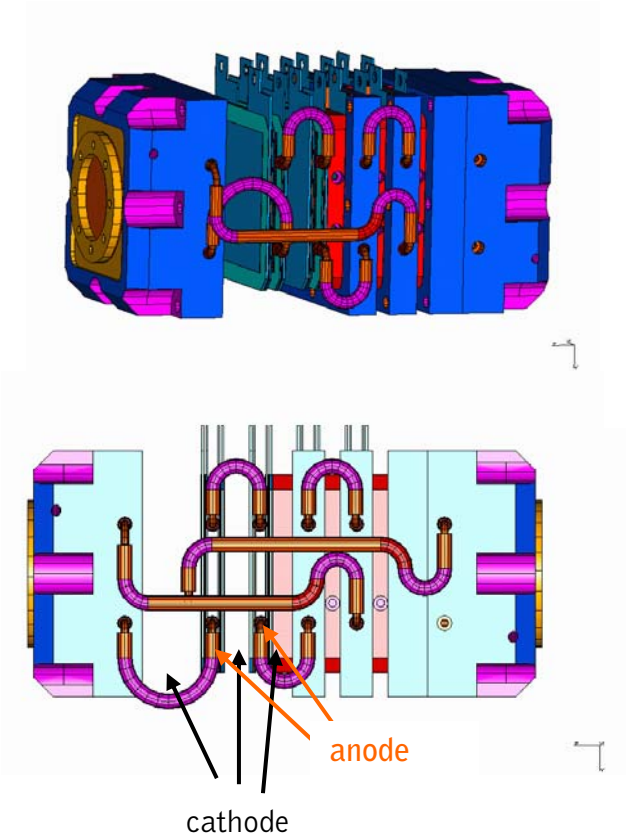


Figure 3.5. The eight-cell stack of the DMBFC.

The first-generation electronic circuit in Figure 3.4a was designed and built as a bachelor's thesis work (Kangasniemi, 2002). The control circuit converted the voltage to 3 V. The electronics were developed further by Salcomp Oy (Fig. 3.4b).

3.2.4 Catalyst – methanol dehydrogenase

Methanol dehydrogenase (EC 1.1.99.8) *Methylobacterium extorquens* is a membrane-associated quinoprotein. Methanol dehydrogenase (MDH) is present in all Gram-negative bacteria capable of utilising primary alcohols and primary formaldehyde. The basic composition of the enzyme normally consists of two identical subunits ($\alpha_2\beta_2$) and contains two molecules of pyrroloquinoline quinone (PQQ), a redox cofactor (Fig. 3.6). The molecular weight of an α-subunit is approximately (MW=66 kDa), and the PQQ is part of this subunit. Each α-subunit contains one calcium ion, which is essential for maintaining the PQQ in its active configuration in the active site (Peihong et al., 1995; Anthony, 1996). The β-subunit is smaller, having an MW of 8.5 kDa. The prosthetic group has a distinctly high midpoint redox potential, -150 mV vs. SCE, compared to the NADH/NAD^+ potential of –560 mV versus SCE at pH 7 (+90 mV and -320 mV vs. SHE at 25°C and pH 7, respectively; Matsushita et al., 2002). PQQ enzymes are particularly

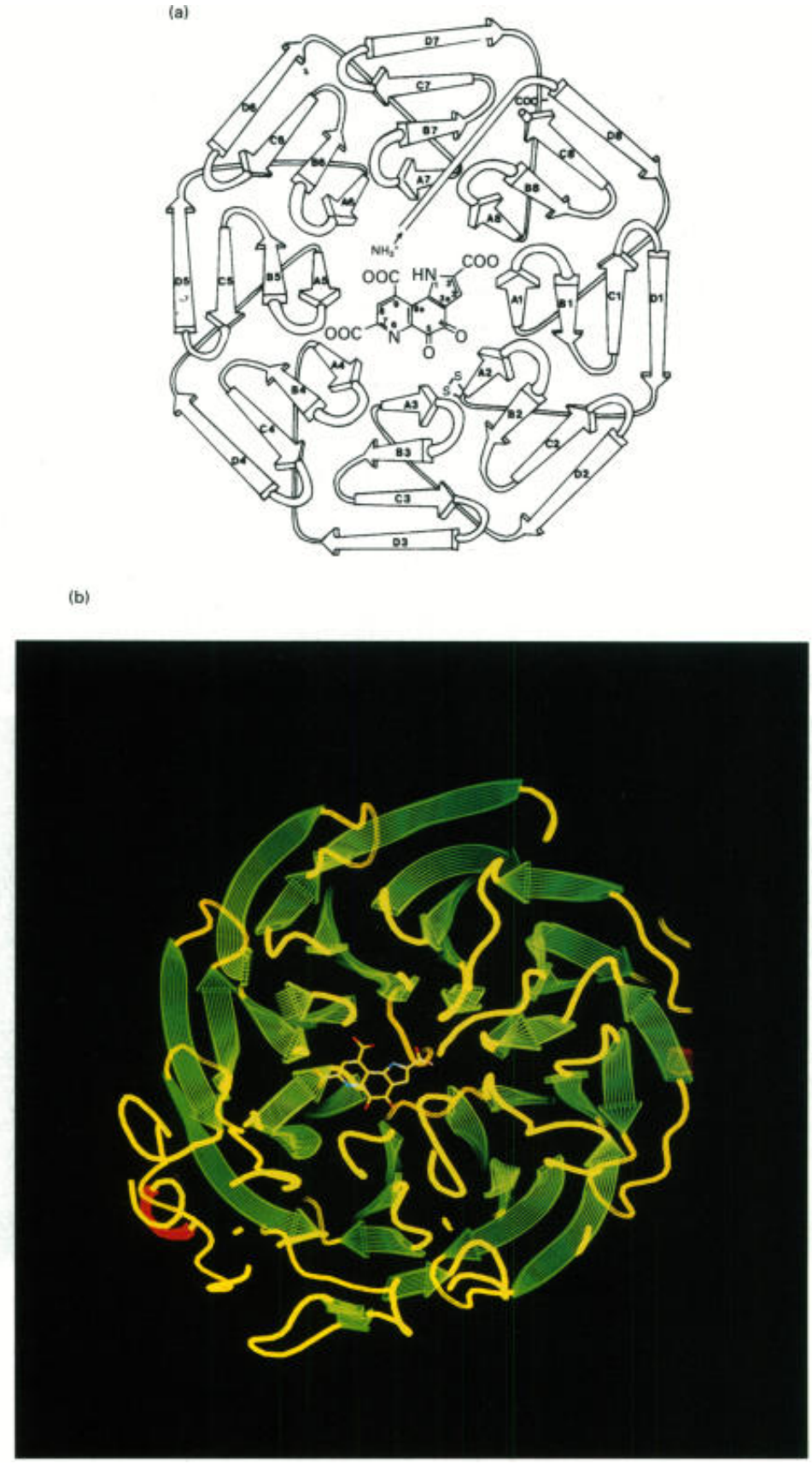


Figure 3.6. Structure of methanol dehydrogenase; the PQQ unit is located in the centre of the enzyme (chemical structural formula in the middle) Reproduced with permission, from Anthony, Ghosh, and Blake, (1994), Biochemical Journal, 304, 665-674.

interesting as biofuel cell catalysts because the cofactor is bound to the enzyme. Thus, the addition or generation of a separate cofactor, such as NAD or FAD, is not necessary.

Two general mechanisms for the methanol oxidation by methanol dehydrogenase have been proposed (Xia et al., 1996). One involves the covalent addition of the methanol to the PQQ prior to its reduction, with the formation of a hemiketal intermediate. The other proposed mechanism is a direct hydride transfer from the alpha-methyl of methanol to the PQQ. For both mechanisms, acid and base catalysis by the enzyme may take place. The base is needed for the abstraction of a proton from the hydroxyl of the substrate. An attack on the carbon 5 of the PQQ would then occur either by the binding of the substrate via an oxyanion intermediate or by direct hydride transfer from the methyl group of the methanol. For the direct hydride transfer the following steps occur: (1) association of methanol to active site calcium ion; (2) deprotonation of calcium-bound methanol by an active site Asp simultaneously with (3) hydride transfer from calcium-bound substrate to the carbonyl at the carbon 4 position and formation of a calcium-bound formaldehyde, and (4) the release of formaldehyde from the active site of the enzyme. Anthony (2004) suggests that the present knowledge of the mechanism supports hydride transfer and that the same mechanism is valid for all the enzymes of this group. An ammonium ion is needed for the expression of MDH activity.

Table 3.4. Properties of the methanol dehydrogenase preparations of Alkomohr Biotech Ltd utilised in the research.

pH optimum	9.5
Temperature optimum	~40 °C
Half-life of enzyme activity at +4°C[1]	70 d
Half-life of enzyme activity at room temperature	30 d
Methanol tolerance	≤ 20% (Up to 40%)

[1] with stabiliser (glycerol)

The properties of the MDH preparations utilised in the study are given in Table 3.4 above. The optimal pH of MDH was 9.5; it also showed some tolerance to a drop in pH without a complete loss of activity. Additionally, the enzyme was found to be remarkably stable. The half-life of enzyme activity was 30 days at room temperature and 70 days in a refrigerator. Methanol concentrations of 20% or less were utilised in testing, but the enzyme tolerated concentrations as high as 40%.

The standard method of activity determination (Day and Anthony, 1990) gives quite low specific activity for the MDH. In a fuel cell application, activity values as high as possible are naturally preferred. On the other hand, the standard activity definition may not give completely accurate information on the enzyme's performance in a fuel cell process. Standard activity assay is designed to show the top value of activity for only a short period of time. When considering a practical biofuel cell, it is far more important to have an enzyme which is stable and functions for long periods of time than to have high initial activity values. During the study the long-term activity of the MDH was observed to be 10% of the standard activity measurement value in fuel cell conditions. In addition, one batch of enzyme was able to operate the fuel cell for two weeks with pH control.

3.2.5 Mediator - TMPD

As mentioned above, the redox reaction occurs in the activity centre of the enzyme. In the case of MDH, the activity centre, i.e., the PQQ prosthetic group, is located in the inner part of the enzyme. Because of the location of the activity centre, placed inside an otherwise non-conductive enzyme molecule, there exists a restriction on the electron transport to the actual electrode (see Section 2.3.2). Therefore, a mediator is required to enhance the electron transport to the anode electrode. An appropriate mediator for MDH has to have a higher standard potential than the PQQ molecule (E^0(PQQ) = -150 mV vs. SCE pH 7; Matsuhita et al., 2002). Nevertheless, it is not beneficial to have too large a potential difference between the mediator and the activity centre. This would lead to poorer performance because of the increased overall potential of the anode and consequently reduced overall voltage of the fuel cell. The mediator also has to have good stability in the fuel cell conditions to ensure the system's operation over a long period of time. The mediator itself is not consumed in the reaction.

For the DMBFC the mediator of choice was N,N,N',N',-tetramethyl-p-phenylenediamine (TMPD). The standard potential of TMPD is +37 mV (SCE at pH 7). However, the high pH of the anodic solution changes the potential of the mediator from the reported value to -55 mV (measured at pH 10 against SCE). TMPD has three oxidation states: R (colourless), $S^{\cdot+}$ (blue), and T^{++} (deep blue) (Fig. 3.7). The basic form R is the reduced form of TMPD and the cations S^+ and T^{++} are oxidised forms. The second state, $S^{\cdot+}$, is the well-known reagent Würster's Blue. The selected mediator has a high reaction constant with reduced MDH (Frank et al., 1988). The ability of TMPD to readily release electrons of the reduced form to many electrode materials has been proven experimentally.

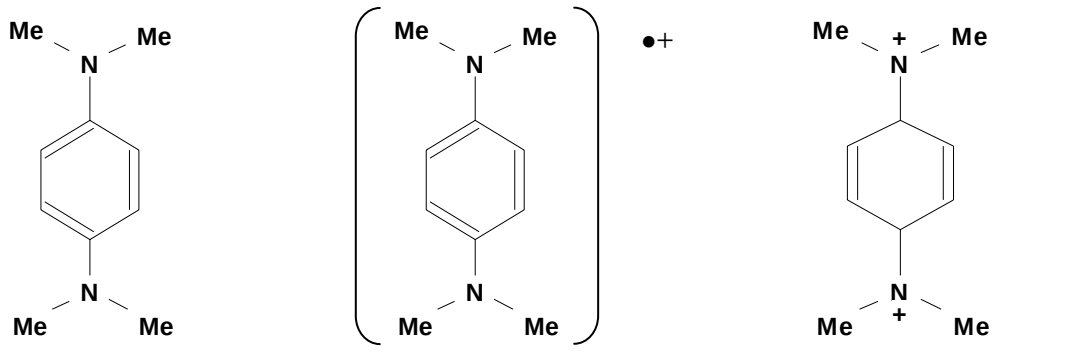


Figure 3.7. The three redox states of the mediator TMPD.

TMPD can oligomerise in aqueous solutions, i.e., the molecules form short chains ($(TMPD)_n$, n=2-10). Like most mediator substances, it is more stable in organic solvents. The experiments support this; TMPD is not stable in inorganic buffers such as a phosphate buffer. Fresh TMPD solution contains all three oxidation states of TMPD, the colourless form R being the dominant one. Michaelis et al. (1939) suggested that the instability is caused by dimerisation or oligomerisation of the TMPD molecules. Frank et al. (1988) reported that the oxidised forms, S^+ and T^{++} radicals, especially T^{++}, were much less stable when compared to the R

form. The Radical T^{++} dimerised or oligomerised, rendering the colour of the solution light brown in time. Oligomerisation is an irreversible reaction and permanently deactivates the mediator.

3.2.6 Stabilising agents

A stabiliser is a chemical compound which improves the stability of the mediator. Peihong et al. (1995) reported that some oxides and salts have a beneficial effect on the electron transfer between the mediator and anode current collector. These chemicals may function as catalysts or by modifying the surface charge. Three metal oxides (TiO_2, Al_2O_3, Fe_2O_3) and one ferrocyanide salt ($K_3Fe(CN)_6$) were tested for their stabilising effect.

3.3 Experiments

3.3.1 Electrical characteristics and the test set-up

The electrical characteristics were determined with the following system: the fuel cell, unit cell (Fig. 3.8), or stack (Fig. 3.9), was connected to a connection board and an adjustable resistor. The open circuit voltage (OCV) was determined after the voltage curve had stabilized. The short circuit current was measured by loading at 2 Ω for 2 minutes. The internal resistance was defined by the Equation 3.3 below. The OCV is designated as U_{OCV}, voltage under load U_L, R_L is the load resistance and U_L is the load voltage at the end of the first minute of discharge.

$$R_{IN} = R_L * (U_{OCV} - U_L)/U_L \qquad (3.3)$$

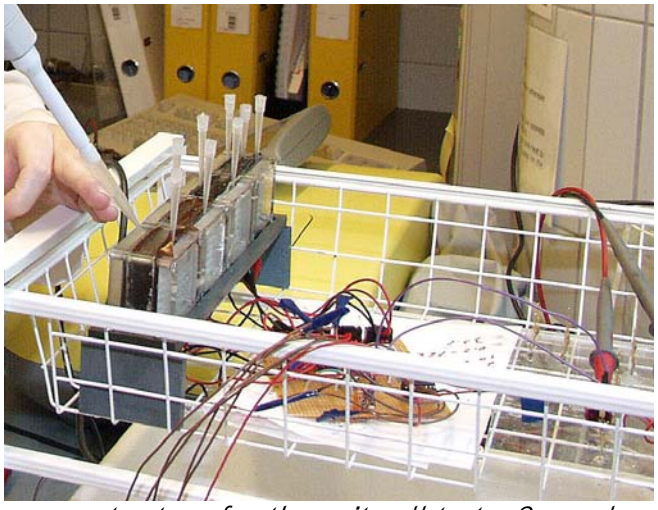


Figure 3.8. Measurement set-up for the unit cell tests. Several unit cells in testing. Water-filled pipette cones are used as caps.

The performance tests to determine capacity, power density and current density were performed under constant load. The data acquisition was performed with a Fluke 189 multimeter (Fluke Corp., USA). The system set-ups are illustrated in Figures 3.8 and 3.9.

Figure 3.9. Test setup for the DMBFC stack.

The connection board shown in Figure 3.9 above was built for the stack testing. It made possible the measurement of individual cells and two to eight cells connected in parallel. The schematic is presented in Figure 3.10.

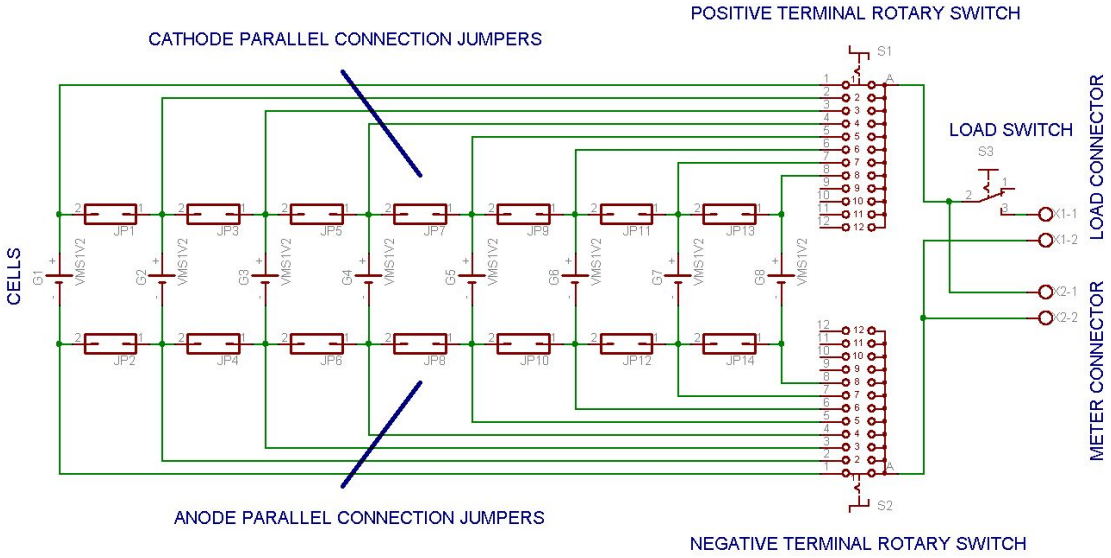


Figure 3.10. Schematic of electronic connection board utilised in stack testing (on a larger scale in Appendix 1).

A novel protocol of loading for the DMBFC was created and tested by Kielosto (2005). The principle was to connect the cells in parallel, load one cell at a time, and convert the voltage with electronics.

3.3.2 Experiments related to MDH

Estimation of the rate of fuel utilisation, η_{FUEL}, was based on the coulombic output of the DMBFC over a period of time and the coulombic value of the reacted methanol. Since the laboratory equipment did not allow recording of the changes in the methanol concentration of the anode solution, the amount of methanol consumed in the reaction had to be defined indirectly.

Three buffer solutions were tested for their buffering capacity by titration with 3.98 M formic acid (100 mM phosphate buffer, 200 mM phosphate buffer and 100 mM sodium borate).

3.3.3. Experiments related to TMPD

The dependence of mediator and current output was determined by measuring the current output with different concentrations of TMPD. The tests were performed with 0-10 mM TMPD solution in the unit cell.

The effect of pH on the absorbance of the mediator was tested by measuring the absorbance of 0.5 mM TMPD in phosphate buffer solution at pH values 7-11. The stability of TMPD was tested by recording the absorbance change at 563 nm of a 0.5 mM TMPD solution subjected to daylight and atmospheric oxygen. Conditions in a biofuel cell were simulated by testing the effect of continuous charge-discharge cycles on the mediator solution. The experiment was performed with the closed cathode unit cell; the anodic solution contained 0.5 mM TMPD buffer solution without any enzyme. The TMPD solution was discharged by short-circuiting for 90 minutes and charged by applying -400 mV voltage to the cell for 90 minutes repeatedly. The absorbance of the TMPD solution was measured every 10 minutes. All the absorbance measurements were performed with a spectrophotometre (Ultrospec 3100, Biochrom, UK).

The stabilizing effects of titanium dioxide, TiO_2, aluminum oxide, Al_2O_3, iron oxide, Fe_2O_3 and potassium ferrocyanide, $KFe(CN)_6$ were also tested also with the closed cathode unit cell. The chemical was mixed into the graphite paste (approx. 0.2 % w/w). The reference anode was prepared by coating a graphite foil with a carbon paste with no additives.

Chapter 4

POWER GENERATION IN A DIRECT METHANOL BIOCATALYTIC FUEL CELL

4.1 Generation of electromotive force in the DMBFC

The purpose of this chapter is to identify the different factors affecting the power generation of the DMBFC. The principle of energy generation in a biofuel cell was explained in Chapter 2. The operational principle of the DMBFC is based on the enzymatic breakdown of methanol by the methanol dehydrogenase from *Methylobacterium extorquens* and mediated electron transfer by TMPD. Equations 4.1 to 4.6 describe the complete chain of reactions occurring inside the fuel cell.

Methanol dehydrogenase is theoretically capable of releasing four electrons per methanol molecule in two steps (Eq. 4.1-4.2). The first reaction is more dominant than the second one. The released electrons are attached to the activity site of MDH, thus changing the oxidation state of the enzyme. The enzymes do not generally readily release electrons to artificial electrode surfaces (Senda, 1990; Ikeda and Kano, 2001; Heller, 1990). The enzyme is a large molecule and if the activity centre is located in the inner part of the molecule, the distance between the electron and the electrode surface is large. A mediator is therefore needed to enhance the transfer rate. As described in Chapter 2, the mediator shuttles between the enzyme and anode electrodes, transporting electrons (Eqs. 4.3-4.4). On the cathode the electrons which have travelled through the external circuit are combined with hydrogen ions (or other positive ion species) that have travelled through the membrane and oxygen, producing water (Eq. 4.6). As for a closed cathode containing potassium permanganate, the end product is manganese dioxide (Eq. 4.7).

Enzymatic breakdown of the fuel in the anode chamber

$$CH_3OH + MDH_{OX} \longrightarrow CH_2O + 2\,H^+ + MDH_{RE} \quad (4.1)$$

$$CH_2O + H_2O + MDH_{OX} \longrightarrow CHOOH + 2H^+ + MDH_{RE} \quad (4.2)$$

The mediator captures electrons from the enzyme's activity centre (i.e. from the reduced enzyme)

$$MDH_{RE} + TMPD_{OX} \longrightarrow MDH_{OX} + TMPD_{RE} \quad (4.3)$$

On the surface of the anode electrode the electron is released from the mediator

$$TMPD_{RE} \longrightarrow e^- + TMPD_{OX} \quad (4.4)$$

Cathodic reactions when the terminal electron acceptor is a chemical oxidant

$$3e^- + KMnO_4 + 4H^+ \rightarrow MnO_2 + K^+ + 2H_2O \quad (4.5)$$

Cathodic reactions when the terminal electron acceptor is atmospheric oxygen

$$O_2 + 4\,e^- + 4\,H^+ \longrightarrow 2\,H_2O \quad (4.6)$$

The multistep reaction of fuel oxidation increases the number of limiting factors in the electron transfer. Therefore it is necessary to examine each step of the electron flow from the fuel to the oxidant in order to reveal the bottlenecks in the electron transfer reaction.

The theoretical maximum current output for MDH can be determined as in Section 2.3.3 (Eqs. 2.9-2.10). As mentioned above, the number of electrons released per methanol molecule is four. Therefore the theoretical maximum current is 6.4 mA per unit of enzyme activity.

1 IU of MDH releases 4 µmol of electrons per minute; thus $n(e^-) = 4$ µmol and $t = 60$ s

$$Q = nF = 4 \times 10^{-6} \text{ mol} \times 96485 \text{ C/mol} = 38.6 \text{ C}; \; F = 96485 \text{ C/mol} \quad (4.7)$$

$$I = Q/t = 6.4 \text{ mA}; \; t = 60 \text{ s} \quad (4.8)$$

However, the maximum current output can usually be achieved only momentarily, if at all, and many enzymes usually function at lower rates than defined by the standard activity measurement.

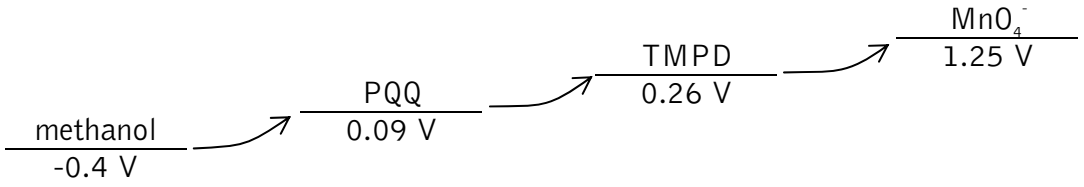


Figure 4.1. Proposed path of the electron transfer from the fuel to the oxidant with potential change (standard potentials at pH 7 vs. SHE).

Figure 4.1 illustrates the proposed pathway from the fuel, methanol, to the oxidant, potassium permanganate. The theoretical open circuit voltage for methanol|potassium permanganate fuel cell is 1.65 V.

4.2 Phases of energy generation in the DMBFC

4.2.1 Flowchart of the process

Figure 4.2 describes a model of the direct methanol biofuel cell on a general level. In order to study the effect of each mechanical or chemical factor on the power generation, the process is divided into five separate phases. Subprocess 1 involves the oxidation reaction of the fuel in which electrons and protons are released and a byproduct, formate, formed. Subprocess 2 consists of the electron transfer to the anode electrode surface. Subprocess 3 involves the proton-permeable membrane, which separates the anode and the cathode. Subprocess 4 is electron transfer to the cathode electrode surface and Subprocess 5 the reduction reaction of the oxidant. The terminal electron acceptor can be either atmospheric oxygen, also involving the diffusion of the oxygen (5a) or a chemical oxidant (5b), if a so-called closed cathode is applied. The electronics are denoted as Subprocess 6.

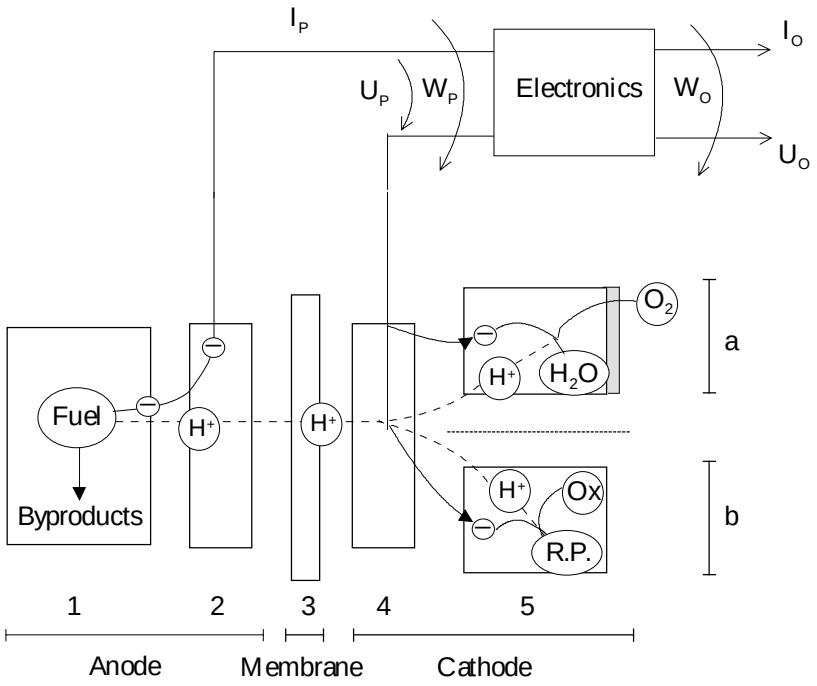


Figure 4.2. Process model of the direct methanol biocatalytic fuel cell.
1: Oxidation of the fuel; 2: Electron transfer to the anode electrode surface; 3: Membrane processes; 4: Electron transfer to the cathode electrode surface; 5: Reduction reaction of the oxidant: a) atmospheric oxygen or b) a chemical oxidant. (R.P. = reduced product)

Table 4.1. Subprocesses of the model in the DMBFC illustrated in Figure 4.2 above.

Subprocess	Description of the step	Section
1	Oxidation of the fuel	4.2.2
2	Electron transfer to the anode electrode surface	4.2.3
3	Membrane processes	4.2.4
4	Electron transfer to the cathode electrode surface	4.2.5
5	Reduction of the terminal electron acceptor: a) atmospheric oxygen b) a chemical oxidant	4.2.6
6	Electronics	4.2.7

4.2.2 Subprocess 1: Oxidation of the fuel

At the anode the fuel, methanol, is oxidised by MDH. The reaction products are formic acid, electrons, and protons. In the first subprocess the possible restrictions are the efficiency of the enzymatic reaction and the access of the enzyme to the fuel molecules. Generally speaking, several factors affect the reaction rate and viability of an enzyme; these include:

- the specific properties of the enzyme: specific activity (activity per unit mass), turnover number (activity per mole of enzyme, number of times the enzyme molecule reacts per second), sensibility to environmental conditions outside the optimum
- the concentration of the fuel and/or products (i.e., substrate or product inhibition)
- the pH of the reaction solution
- the temperature

Although a high specific activity would generally imply a good performance in terms of energy generation, it is more important to have a fairly good turnover number and endurance towards the unnatural conditions in a fuel cell. The properties of the enzyme utilised in this study, methanol dehydrogenase, were described in Section 3.2.4. The standard activity measurement gives quite low specific activity for the MDH, but the standard activity measurement does not give reliable information on the long-term activity level of an enzyme. MDH has proven to be remarkably stable in fuel cell conditions and in storage.

The first potential bottleneck, the efficiency of the enzymatic oxidation of methanol, is affected by the environmental factors: pH and temperature. The optimal pH of methanol dehydrogenase is 9-9.5. At lower pH values the enzyme is inactivated and below a certain pH the activity will not recover as a result of the adjustment of the pH. Thus, the inevitable formation of formic acid is a crucial issue for the energy generation. Elimination of the negative effect of formic acid (pK_a = 3.75) is difficult. Therefore the buffer has to have strong buffering capacity in order to keep the pH of the solution around 8-9 in order to keep the enzyme functional. Additionally, in a fuel cell the buffer has to have good ionic conductivity

values so as to minimise losses in ionic transfer, which in turn increase the internal resistance of the cell.

Generally speaking, temperature has negative effect on the enzyme activity if it falls below or rises above the optimal conditions determined by the characteristics of an enzyme. In cold conditions the reaction rate decreases with decreasing temperature and finally stops completely when the solution becomes frozen. Nevertheless, the enzyme activity can be maintained and restored when it thaws. Heat (50-60 °C), on the contrary, destroys the protein structure of the enzyme and the activity is lost permanently.

The second bottleneck relates to the physical contact of the enzyme molecule and the methanol molecule. The catalyst layer is a complex mixture of graphite particles, binder, enzyme, mediator and stabilisers (Figure 4.3). Due to the physical thickness of the catalyst layer, some of the enzymes are buried too deep in the material to be in contact with the fuel molecules. In order to have all the enzyme molecules active and in contact with the fuel, a perfect monolayer of the enzyme molecules would be required and the environmental factors would have to be under perfect control.

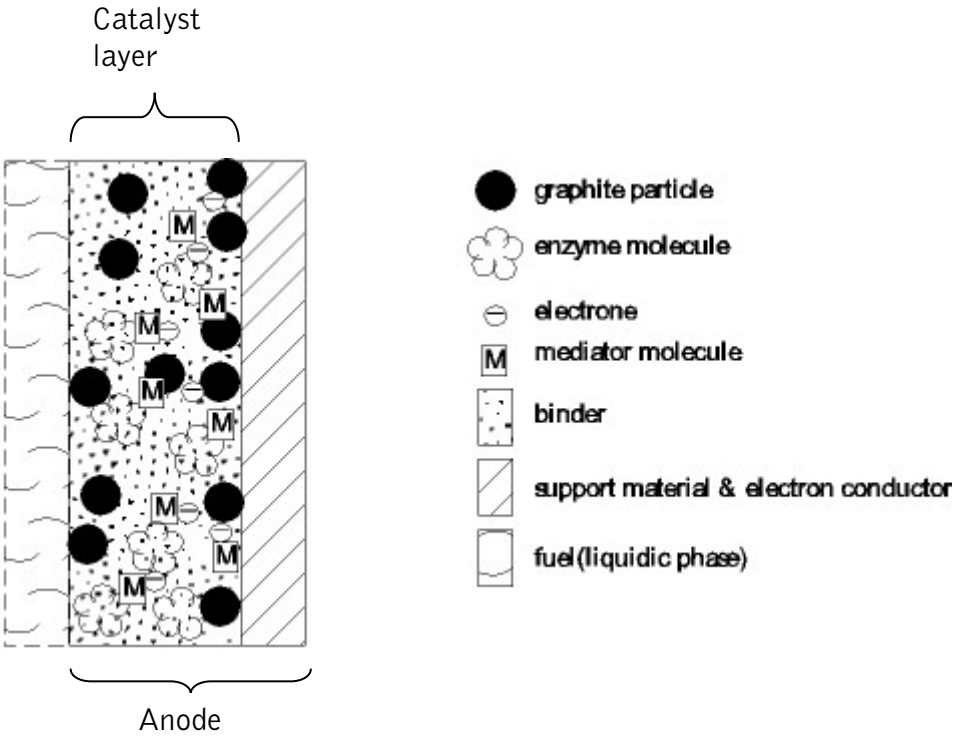


Figure 4.3. Detailed schema of the reactive layer describing the composition of the paste, which contains all the functional elements. The catalyst layer and current collector form the anode electrode.

4.2.3 Subprocess 2: Electron transfer to the anode electrode surface

The second subprocess is the electron transfer from the active centre of the enzyme molecule to the conducting surface of the anodic electrode (i.e., current collector), performed by the mediator. The transfer rate is affected by the concentration of active mediator molecules and the composition of the electron conducting material of the catalyst layer. The paste was prepared manually using basic laboratory equipment. Consequently, there is a risk that the compounds were not evenly dispersed throughout the material. This means that a reduced enzyme molecule may not be in contact with an oxidised mediator molecule and therefore the electron cannot be passed from the enzyme to the electrode. The current collector has to have good conductivity and to be inert towards the compounds present at the anode. It should be mechanically durable, in case it also serves as a support material.

4.2.4 Subprocess 3: Membrane processes

The purpose of the membrane in a fuel cell is to separate the anode and cathode chambers. The most important property of the membrane utilised in a fuel cell is high proton conductivity. Other important factors related to the membrane include minimal crossover of the fuel and mediator from the anode to the cathode. Additionally, if there is a strong pH gradient between the two compartments, as in the DMBFC with a closed cathode, there is a risk of an unwanted backward flow of protons.

4.2.5 Subprocess 4: Electron transfer to the cathode electrode surface

In an oxygen diffusion cathode the composition of the catalyst layer affects the electron transfer rate in the same way as at the anode, as described in Subprocess 2. In the case of a closed cathode, the actual catalyst was not needed. Thus, only the material of the current collector is relevant for the transfer of the electrons passed along the external circuit.

4.2.6 Subprocess 5: Reduction of terminal electron acceptor

Efficient reduction of the oxidant is most important at the cathode. In the case of a DMBFC there are two options: a passive air-breathing oxygen diffusion cathode or a closed cathode with a strong oxidative reagent and no actual catalyst. Generally speaking, a passive cathode (no pumps to increase air flow) is the one best suited for fuel cells intended for mobile electronic appliances.

The oxygen diffusion cathode utilised here was purchased from Gaskatel GmbH (DE) and was a semicommercial product at that time (see Section 3.2.2). Therefore, the important factors, the oxygen diffusion rate, catalyst activity, and electron conductivity, were in order. To create the best possible conditions for the

oxygen cathode it is important to maintain the pH of the cathode electrolyte fairly constant and to have a buffer solution with a high ion conductance.

The principle of the closed cathode was explained in Section 3.2.2. Its operation is based on a strong oxidative chemical. Thus, no atmospheric oxygen or catalyst is needed. The reaction pathway of the potassium permanganate depends on the pH of the solution (see Equations 4.9-4.10 below). A higher potential is reached with a lower pH.

$$KMnO_4 + 5e^- + 8H^+ \rightarrow K^+ + Mn^{2+} + 4H_2O \qquad E_0 = 1.491\ V \tag{4.9}$$

$$KMnO_4 + 3e^- + 4H^+ \rightarrow K^+ + MnO_2 + 2H_2 \qquad E_0 = 1.679\ V;\ pH < 2 \tag{4.10}$$

To conclude, the best functionality of the cathode in both cases is achieved by pH control.

4.2.7 Subprocess 6: Electronics

A practical fuel cell power source usually needs more than one unit fuel cell to generate adequate operational voltage. To make a practical energy generation device from a set of several unit cells (a stack), electronics and an "electricity buffer" – a battery or an (ultra)capacitor – are required for the control and conditioning. The electronics cause a small loss of the primary voltage. In the case of mobile fuel cells, the number of unit cells needed to generate a high enough voltage may be high, making the device too large for the application.

Another aspect is the loading profile of the stack and the connection of the unit cells. A stack is usually made by connecting unit cells in series. The number of unit cells is defined by the target voltage and the voltage generated by the unit cell. A novel idea is to connect the cells in parallel, load one cell at a time, and boost the voltage with electronics. The latter has the advantage that if one cell does not work well it does not bring down the whole system. The control electronics can detect the impaired cell and leave it unused. In addition, there are indications that periodical loading may enhance the performance of the individual cells. This applies to metal catalyst fuel cells as well. In biofuel cells the effect is more pronounced as the cell chemistry is slower than in metal catalyst fuel cells. A short loading period empties the electrons generated at the anode during the recovery period.

Chapter 5

RESULTS AND DISCUSSION

5.1 Outline of the chapter

This chapter describes the main results achieved in the 3-year research. Sections 5.2 to 5.5 report the rate of fuel utilisation and issues related to pH and also the improved stability of the mediator and performance of the cathode. The experiments were performed with the unit cell. The results of stack tests will be described in Section 5.6 and the overall assessment of the results is given in Section 5.7.

5.2 Oxidation of the fuel

To determine the rate of fuel utilisation, η_{FUEL}, of the DMBFC, one has to consider two facts. Firstly, the enzymatic reaction catalysed by MDH releases four electrons from methanol out of the total of six released in complete oxidation. Thus, the maximum fuel utilisation in the enzymatic oxidation of methanol is 67%. The other factor is the efficiency of the enzymatic reaction. The estimation of the efficiency was based on the coulombic output of the fuel cell, Q_{OUT}, and the coulombic value of the reacted methanol, Q_{FUEL}, as defined by Equation 5.3. The risk of the inactivation of TMPD was minimised by having an excess of the mediator. The amount of reacted methanol had to be determined indirectly. Figure 5.1 depicts a load curve of the DMBFC.

The coulombic conversion rate of the fuel, η_{fuel}, is defined by Equation 5.1.

$$\eta_{FUEL} = Q_{OUT} / Q_{FUEL} \qquad (5.1)$$

The coulombic output estimated from the load curve (Fig. 5.1; t = 2-22h).

$$Q_{OUT} = \int_0^t I(t)dt \qquad (5.2)$$

$$Q_{OUT} \approx 600 \text{ C}$$

The electrical quantity of the fuel estimated by the formation of formic acid

$$Q_{FUEL} = N\ n(MeOH)\ F \qquad (5.3)$$

$$Q_{FUEL} = 1100 \text{ C}$$

F = Faraday constant, 96484.56 C/mol
N = 4 electrons / 1 methanol molecule released by MDH

$\eta_{FUEL} \approx 0.55$

The experiments showed that 55-60% of the methanol put into the anode was converted into electricity (η_{FUEL} = 0.55-0.6). On the basis of the result and the fact that the maximal conversion of enzymatic methanol oxidation is 67%, the efficiency of the enzymatic reaction was estimated to be over 70%.

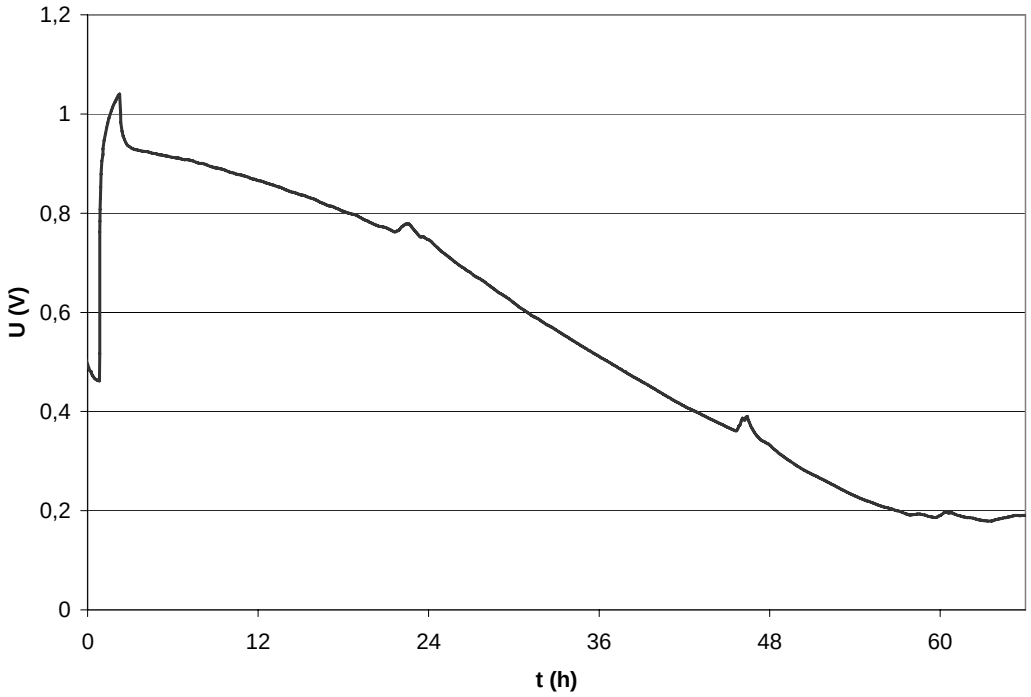


Figure 5.1 Load curve of the DMBFC from April 2003 (R_L = 100Ω, 100 mM sodium borate buffer). The nudges at t = 24 h and 48 h are caused by refreshment of the electrolyte solutions.

In order to function properly, the surroundings of an enzyme have to have a pH suitable for the enzyme. The formic acid produced by the MDH is a classified as weak acid ($K_a = 1.77 \times 10^{-4}$, $pK_a = 3.75$; T = 20 °C). The rate of production of the acid is dependent on the current output; the higher the current, the more acid is formed. Formate is reported to inhibit the activity of MDH of the yeast *Candida boidinii* at a concentration of 7.4 mM (equals 340 mg/l)(Volfová, 1980). A fast drop in pH has been observed in the course of experiments, even though exact measurement of the formic acid generated has not been possible. The small volume of the anode electrolyte increases the challenge. To illustrate this, the anolyte was titrated with formic acid (99.9%). With an anolyte volume of 4.2 ml the pH dropped below 7 with the addition of 6 µl of formic acid (Fig. 5.2). The resulting concentration was 38 mM regarding formate, i.e., 5 times the reported inhibition concentration. MDH does not express activity at a pH of 7 or below. To put the former in perspective and in relation with the DMBFC, the formation of the acid can be roughly estimated on the basis of the reactions presented above (see Section 4.1) and a few assumptions. Half of the electrons observed as current are derived from the second reaction, assuming that the formaldehyde is completely oxidised to formic acid. With ideal conversion, the inhibition limit is reached in a little over

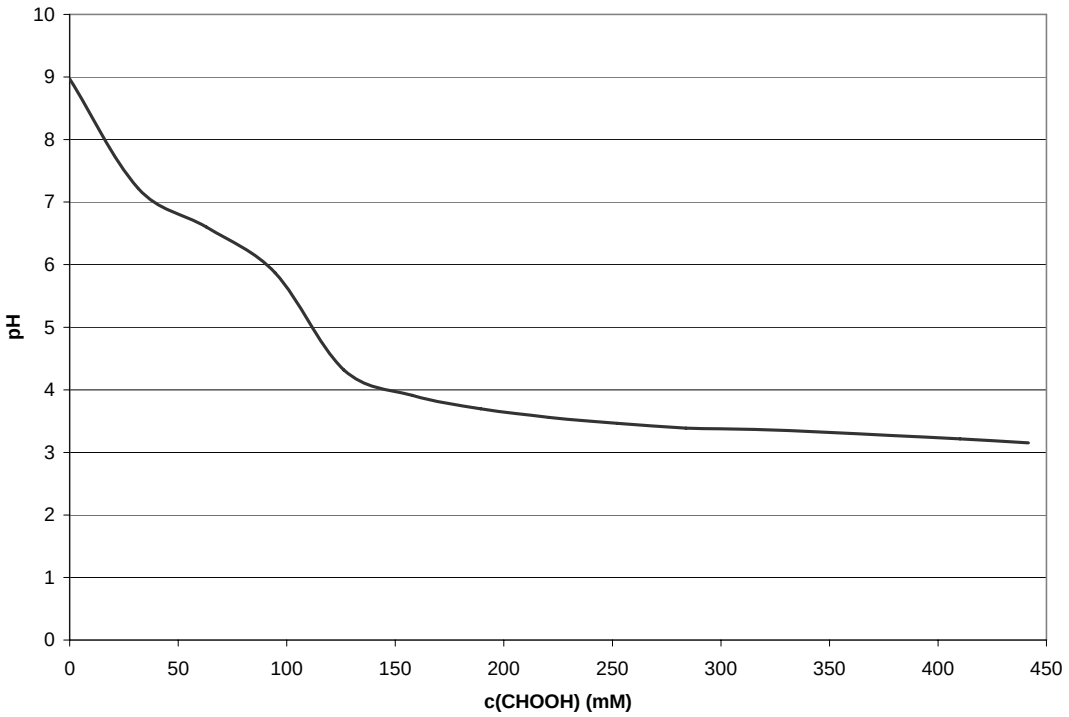


Figure 5.2. Buffering capacity of 200 mM phosphate buffer at changing concentration of formic acid (99.9% (26.5 M) formic acid, volume of the buffer 4.2 ml).

one hour (Fig. 5.3). If one considers enzymatic conversion to be 50%, it still takes less than three hours to reach the limit, according to the estimate.

Assumptions:

i. Constant current 10 mA
ii. 4 e^- released per methanol molecule in two steps (Eqs. 4.1-4.2)
iii. Reaction rates of formaldehyde and formic acid are equal
iv. The molar formation ratio of CHOOH:e^- is 1:2
v. Anode volume is 16 ml
vi. Perfect conversion

$$I = Q/t = nF/t \Rightarrow n/t = I / F ; \quad (5.4)$$

where F = 96485 C/mol and n the amount of substance

Amount of electrons per time unit to create 10 mA current

$$n/t(e^-_{10\ mA}) = I / F = 1.036 \times 10^{-7}\ mol/s \quad (5.5)$$

$$n/t(e^-_{CHOOH}) = 0.5 \times n/t\ (e^-_{10\ mA}) \quad (5.6)$$

$$n/t(CHOOH) = 2.59 \times 10^{-8}\ mol/s \quad (5.7)$$

The molar production rate of formic acid, c/t(CHOOH) is given by Equation 5.8 and plotted against time in Figure 5.3.

$$c/t(CHOOH) = 1.62 \times 10^{-6}\ mol/l{\cdot}s \qquad (5.8)$$

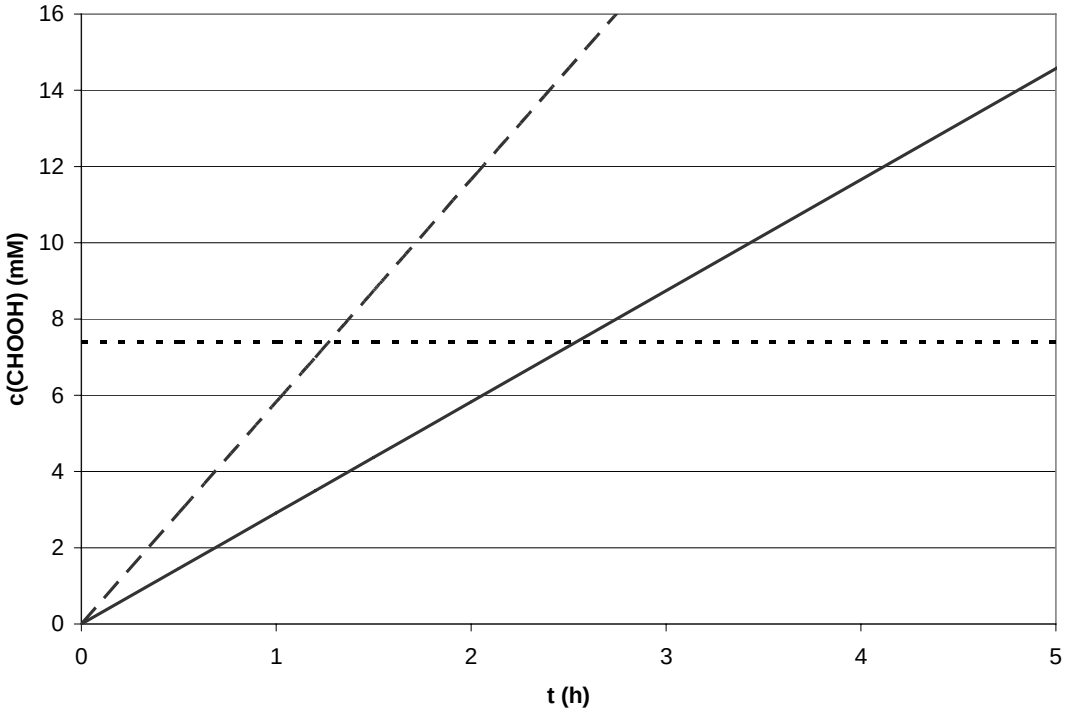


Figure 5.3. Rough estimate of acid production with a constant current of 10 mA; perfect conversion (broken line) and 50% conversion (solid line). The dashed horizontal line illustrates the reported inhibition level of formic acid.

Even though the estimation is only indicative, it does illustrate the seriousness of the need for pH control or the elimination of the acid produced in the DMBFC. Experiments have repeatedly proven that during test runs the pH of the anolyte has to be adjusted on a regular basis. The interval depends on the utilised buffer solution and the current produced by the cell. In the DMBFC stack the adjustment was made every 5 hours. In the case of the unit cell, the interval was longer, in the best cases once a day. To keep the pH of the solution above 8-9 and the enzyme functional, solutions of different concentrations were tested for their buffering capacity: sodium borate and phosphate buffers (Table 5.1). Figure 5.4 shows how fast the pH changed with the addition of formic acid. The experiment proved an earlier observation, the sodium borate was clearly the best one. Still, because of the better ion conductivity of the phosphate buffer, it was used in short-term tests and sodium borate in the long-term ones. There is a possibility that the Ca^{2+} ions at the activity centre of MDH will be removed by phosphate ions. Deactivation was not clearly observed in practice, but this is a further reason to utilise the borate buffer in long-term runs.

Table 5.1. Tested buffer solutions.

100 mM sodium borate	$Na_2B_4O_7{\cdot}10H_2O$ - NaOH
100 mM phosphate	K_2HPO_4 - NaOH
200 mM phosphate	K_2HPO_4 - NaOH

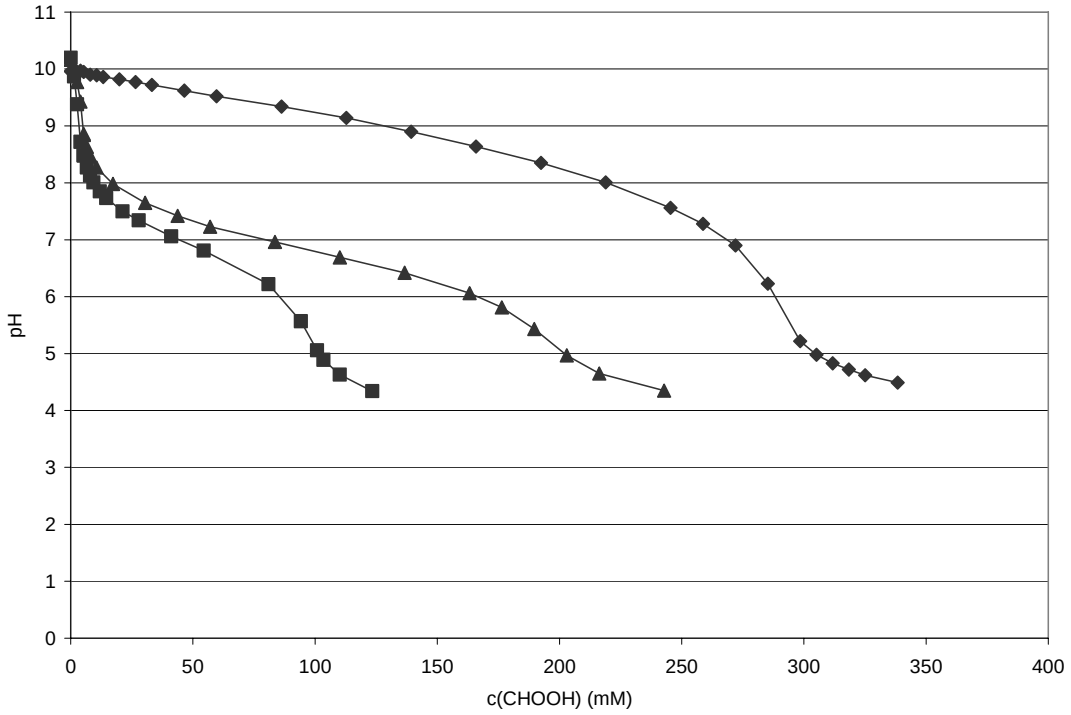


Figure 5.4. Buffering capacity of three buffer solutions tested with the addition of 3.98 M formic acid: ■ 100 mM phosphate, ▲ 200 mM phosphate, and ♦ 100 mM sodium borate (volume of the buffer 30 ml).

Nevertheless, a viable method for the elimination of the acid remains an unsolved challenge. Even the strongest buffer is quickly used up with small anolyte volumes. So, buffering is not a feasible solution for the problem posed by acid production in the long term. All the test runs were made with low enzyme concentrations; to increase current output a higher enzyme concentration is needed but at the same time the production of acid will increase and the buffer capacity will become well below what is needed. The solution to this problem most probably requires the application of active filter materials, which make possible the controlled diffusion of an alkali to the anode chamber or acid flow from it.

5.3 Charge transfer to the anode

Charge transfer to the anode is affected by the activity and stability of the mediator and the conductivity of the catalyst layer. The properties of TMPD as a mediator were explained in Section 3.2.5. As Figure 5.5 shows, the TMPD concentration should be 5-10 mM in order to ensure a good electron transfer rate. Because in an enzymatic fuel cell the pH of the environment is very important for efficient enzymatic reaction, the effect of different pH levels on TMPD was tested (Fig. 5.6). The tests showed that in the pH range of MDH the absorbance was rather low, suggesting that at higher pH values the reduced colourless form of TMPD (i.e., the R radical) is more dominant and the oxidised forms become dominant at a lower pH. This is somewhat contradictory to the conditions dictated by MDH.

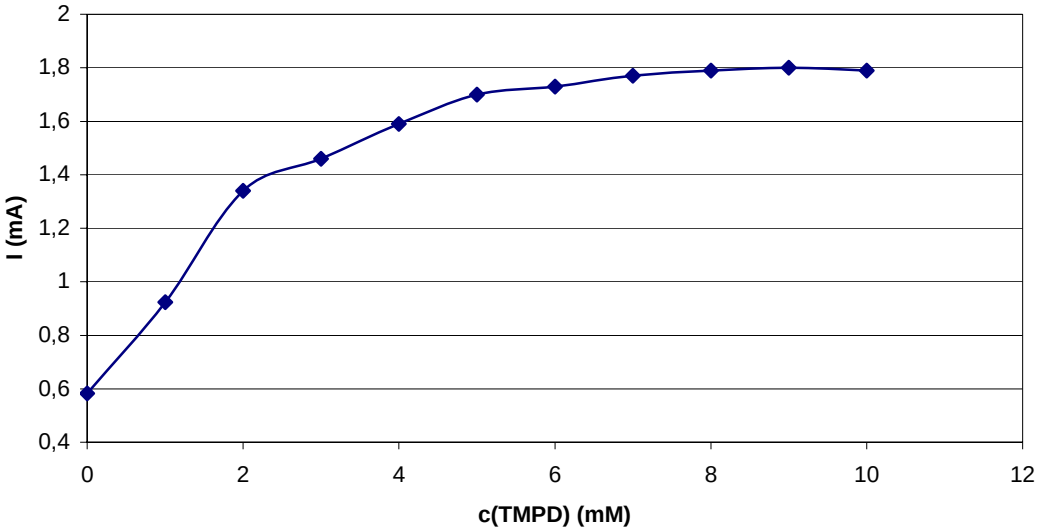


Figure 5.5. Correlation of TMPD concentration and current output.

The oligomerisation of the mediator TMPD was discussed earlier. The role of the mediator is central to the performance of the biofuel cell. As explained, the oligomerisation decreases the rate of electron transfer by reducing the number of active transport molecules and is an irreversible reaction. Additionally, it is known that TMPD – and mediators in general – are more stable in organic solvents than in aqueous solutions such as a phosphate buffer. This was verified by experiment; a colour change from clear to light brown was observed after a few weeks in the TMPD-phosphate buffer solution kept at an ambient temperature. Figure 5.7 illustrates the spontaneous absorbance change.

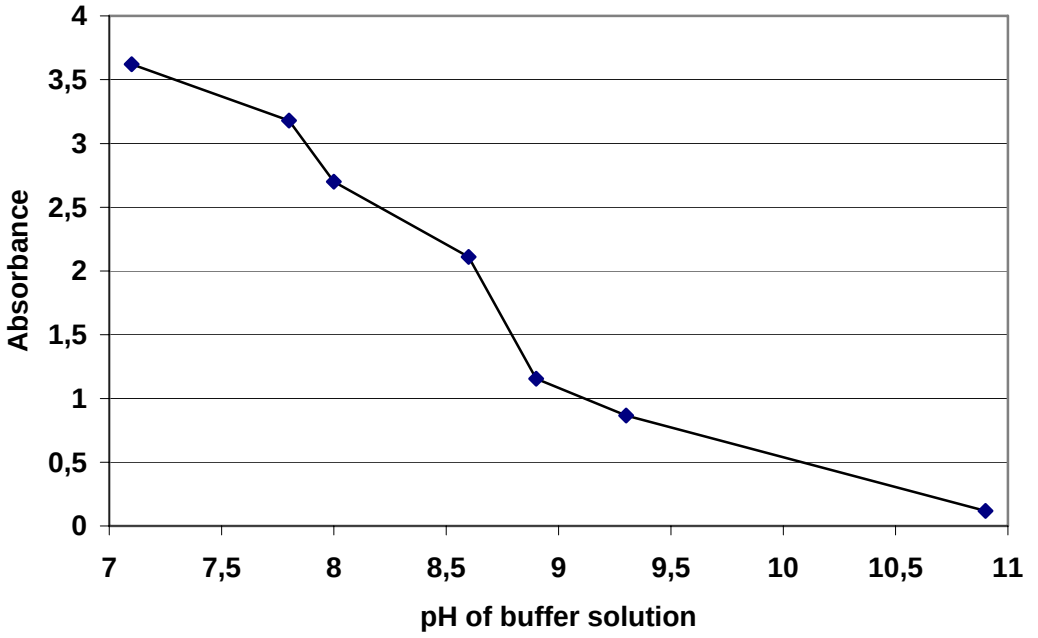


Figure 5.6. Effect of pH change in 0.5-mM TMPD-phosphate buffer solution.

The absorbance of the solution increased in the first hours of the experiment as TMPD was oxidised, changing the solution to deeper blue as a result of the increasing concentration of S^{+} and T^{++} cations. Gradually, the absorbance started to decrease as the colour turned to light brown. According to the literature, the second colour change observed implies the dimerisation or oligomerisation of the radical T^{++} (Michaelis et al., 1939; Frank et al., 1988). Consequently, the effect of fuel cell conditions was simulated by continuous charge-discharge cycles (discharge for 90 min, charge -400 mV for 90 min in phosphate buffer pH 9). The rate of oligomerisation was observed to be accelerated if compared to spontaneous reaction. One possible explanation of the observed phenomenon is that under a heavy current output the concentration of oxidised TMPD, and thus the number of less stable forms of the mediator, increases remarkably. This would explain the increasing rate of oligomerisation.

Figure 5.8 illustrates the decrease in absorbance with time of the measured TMPD solution during repeated charge-discharge cycles. After every charge-discharge cycle the ability of the TMPD solution to take up electrons was less than in the previous cycle. The half-life of TMPD was defined as approximately 10 days in normal conditions (Fig. 5.7), but decreased to less than 5 days in simulated fuel cell conditions (Fig. 5.8).

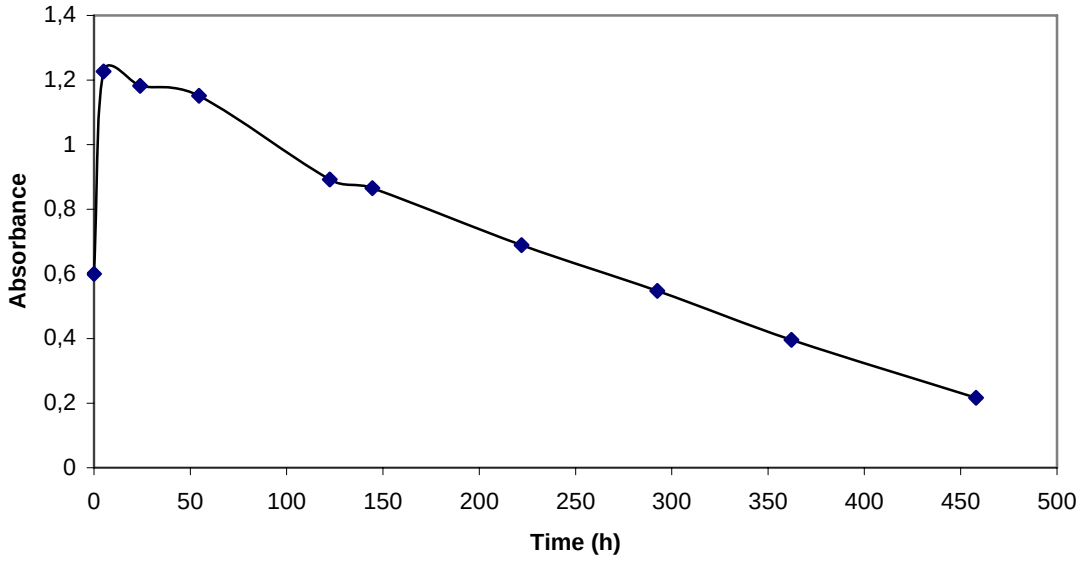


Figure 5.7. Development of the absorbance of TMPD (0.5 mM) in phosphate buffer pH 9 with time (λ = 563 nm). The half-life t½ ≈10 days. (*Zhang, Ranta and Halme, 2006*)

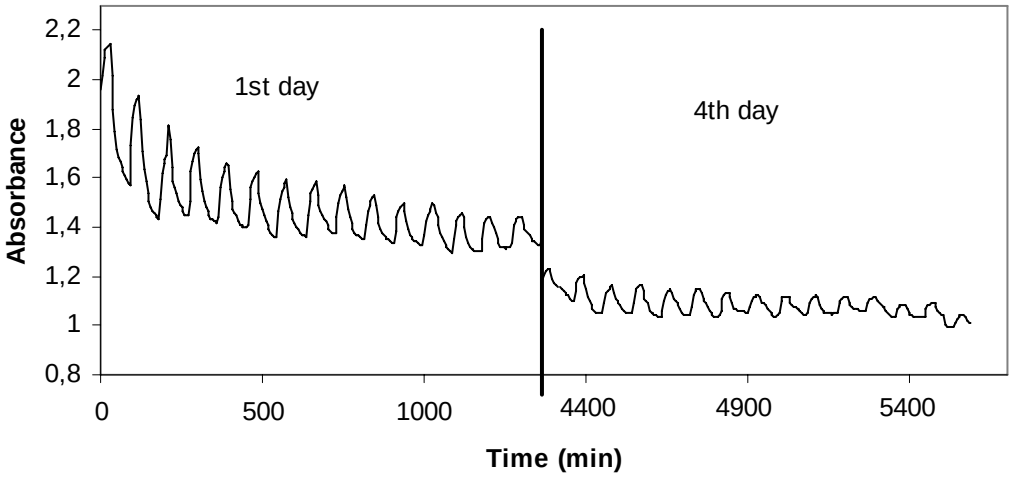


Figure 5.8. Effect of charging and discharging on the absorbance of TMPD during the 4-day experiment (λ = 563 nm, 5 mM TMPD – phosphate buffer pH 9). The half-life t½ ≈ 4 days. (Note: the vertical line symbolises missing data from the second and third day.) (Zhang, Ranta and Halme, 2006)

A positive effect of certain salts and oxides on the lifetime of the mediator has been reported (Peihong et al., 1995). These chemicals are presumed to function as catalysts or by modifying the surface charge. In the DMBFC study three metal oxides and a ferrocyanide salt were tested in the fuel cell set-up with different loading profiles (Table 5.2). The stabilisers were mixed into the anode paste, which also contained the enzyme and mediator. The amount used was approximately 0.2% w/w. Each stabiliser expressed a clear positive effect compared with the reference anode, the best being titanium dioxide. Moreover, visual observation confirmed that the colour of the anode solution during the process did not change to brown, as previously. The colour switched between dark blue and colourless with titanium oxide and aluminum oxide; with ferric oxide and the salt it was between deep orange and colourless. Thus, one can conclude that the salts that were tested stabilised the TMPD and slowed the rate of dimerisation or oligomerisation.

Table 5.2. Effect of stabilising agents on the performance of the DMBFC (Zhang, Ranta and Halme, 2006).

Loading profile Additives	**1 h-100 Ω load**		**18 h-100 Ω load**		**18 h-2000 Ω load**	
	I (mA/cm^2)	P ($\mu W/cm^2$)	I (mA/cm^2)	P ($\mu W/cm^2$)	I ($\mu A/cm^2$)	P ($\mu W/cm^2$)
Reference	0.02	0.67	0.009	0.13	3.14	0.32
TiO_2	0.028	1.27	0.013	0.26	4.99	0.79
Al_2O_3	0.025	0.98	0.012	0.23	4.59	0.675
Fe_2O_3	0.027	1.2	0.011	0.19	4.76	0.73
$K_3Fe(CN)_6$	0.027	1.18	0.013	0.26	4.68	0.7

In a previous study of microbial fuel cells, a method to monitor the state of the biological process by means of the colorimetric measurement of the colour changes of HNQ was reported (Halme et al., 1998). The DMBFC system had the same property: limitations in electron transfer between the anode and the enzyme, as well as the deactivation of the mediator, can be visually monitored. For example, when the coloured form (i.e., oxidised form) appears under heavy loading, it implies that the enzymatic reaction is not fast enough and consequently the oxidised form of the mediator starts to accumulate.

5.4 Material issues

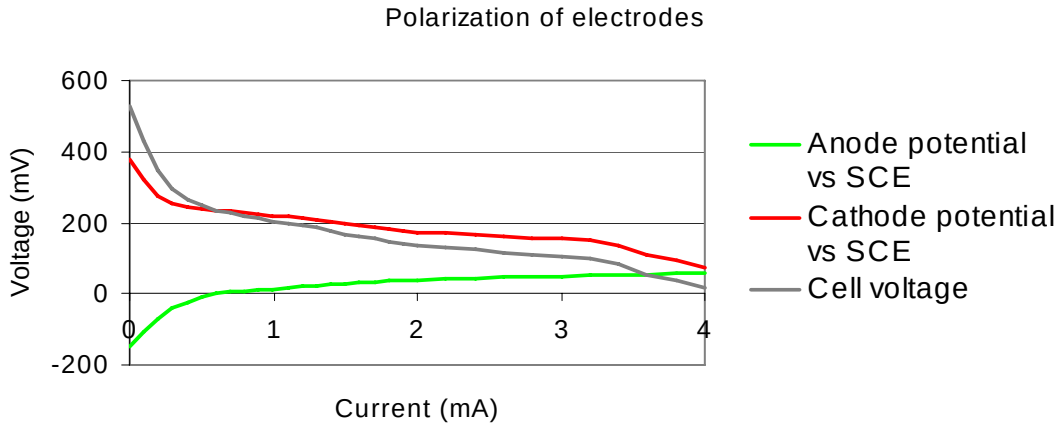
The mechanical structure and, at the same time, the current collector of both electrodes was graphite foil in the unit cell (Fig. 3.3). Stainless steel plates were utilised as the anode and graphite foil as the cathode electrode materials in the stack (Fig. 3.5). Graphite foil is an inert material with a high conductance that tolerated the reaction conditions well. The downside of the use of this foil is its low mechanical durability. Thin foil is preferred in thin structures, but it is also easily broken. Electrical connections required support material such as conducting tape. The brittleness of graphite was especially inconvenient in the DMBFC stack.

The BDH membrane is a standard cation exchange membrane that has been used in a demonstration biofuel cell set-up by NCBE (UK). The BDH membrane worked better in the DMBFC than a Nafion membrane, which was in use in the early phase of the DMBFC study. Nafion had to be discarded because the mediator was absorbed by it. The BDH membrane was not able to prevent the migration of the small mediator molecules, but the crossover did not cause short-circuiting. Instead, it slowly reduced the performance by lowering the concentration of the mediator. The BDH membrane is also quite thick in this context (wet thickness 118-120 µm). So it was not an optimal material, but functional for the early phase of the development process. Material issues need to be readdressed on an ongoing basis.

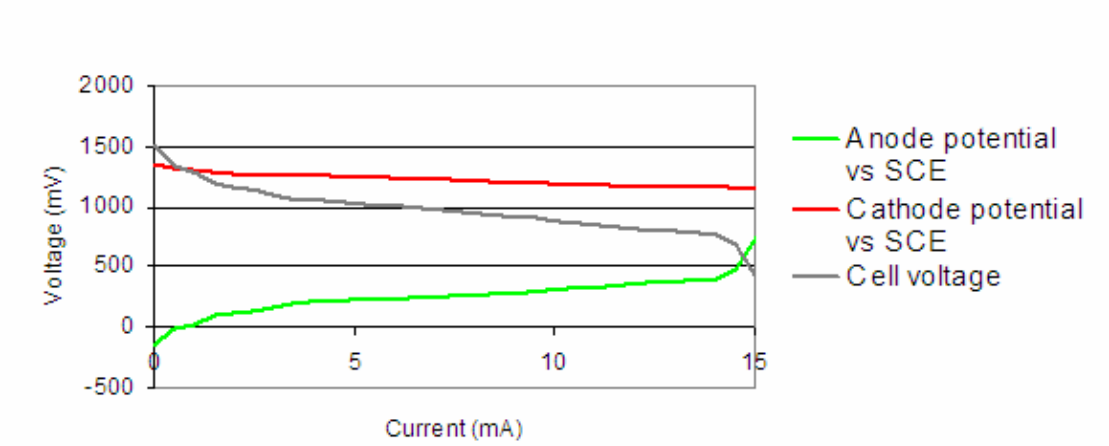
5.5 Reduction of the oxidant

The oxygen diffusion cathode was found to limit the performance of the DMBFC. The cathode potential dropped at low current values (less than 1 mA), and the overall potential was poor as well (Fig. 5.9a). In the long run one explanation is the low pH of the catholyte, which slowly dissolved the silver-based catalyst. This cathode material was designed for alkaline conditions. However, even in alkaline conditions the oxygen cathode did not generate a large enough potential difference with the MDH anode to support the production of current. The potential difference was around 100 mV.

The introduction of the closed cathode increased the cell potential remarkably. An improvement was expected because potassium permanganate is a strong oxidant. Experiments showed that the limitation was transferred from the cathode to the anode with a closed cathode biofuel cell. As Figure 5.9b illustrates, at 14 mA the anode operation is no longer sufficient.



a.



b.

Figure 5.9. The polarisation curves of the oxygen diffusion cathode (a.) and the closed cathode with potassium permanganate (b.).

The experimental results implied that of the two possible reactions of potassium permanganate depicted in Section 4.2.6, the second one, with higher potential, dominates. This conclusion was drawn from of the formation of a mud-like precipitate (MnO_2). Because all of the oxidant is stored in the fuel cell structure, it has to be renewed every once in a while. This is the penalty for better performance. In the biofuel cell set-up being presented, 80% of 16 ml of 0.4 M potassium permanganate solution will be used in about 80 hours at a constant current of 5 mA. Thus, the catholyte has to be changed every three days.

The downside of the utilisation of potassium permanganate is that the pH of the catholyte should be kept very low, around 2, to get the highest potential value (Eqs. 4.9-4.10). Because the anode is at a high pH there will be a great pH gradient between the two electrodes. In principle this is good, because it helps to increase the overall potential of the cell. In practice the large pH difference can create a small proton flow to the anode through the membrane. Nevertheless, a pH change

of a highly alkaline and acidic chamber separated with the BDH membrane was not observed in a test. As described above, maintaining the pH of the anolyte in the appropriate range for the enzyme is already difficult because of the acid produced in the enzymatic reaction. Another impractical property is the corrosiveness of permanganate. It puts a strain on the component materials and the sealing of the device. Furthermore, permanganate is toxic to aquatic life. Thus the utilisation of permanganate in a consumer device is not recommendable, even though it is useful in laboratory testing.

5.6 Stack tests

The stack-structured DMBFC (see Section 3.2.3) was designed to generate around 100 mW (1.7 mW/cm^2) at 0.5-0.7V using the enzymatic oxidation of methanol and a closed cathode. The enzyme load was 24 units, which in an ideal case would correspond to approximately 75 mW (1.3 mW/cm^2) at 0.5 V. The expected performance was: OCV 1.2-1.4 V, maximum steady current for the first two days 25-30 mA (0.43-0.52 mA/cm^2), long-term steady current 12-18 mA (0.21-0.31 mA/cm^2), maximum steady power 18-22 mW (0.31-0.38 mW/cm^2), and long-term power 9-13 mW (0.16-0.22 mW/cm^2).

The circulation of the anodic and cathodic reaction solutions was arranged with a manual pump. The pump was not able to create sufficient liquid flow through the chambers. Air bubbles were the biggest reason in the case of the anode chambers. At the cathode the precipitate that formed clogged the connecting pipes and the surface of the current collector. In addition, the rubber diaphragm of the pump did not tolerate the oxidant permanganate. After some repairs, the circulation of the cathodic electrolyte was performed with a peristaltic pump. There was gas production in the cathode, which created extra pressure and pushed the catholyte out.

The strong oxidant also created material problems. The plastic utilised could not stand the corrosiveness of the oxidant. Fracture lines were formed, especially in the thinner parts. The declining OCV depicted in Figure 5.10 below implies internal leakages between the anode and cathode, causing a chemical short circuit.

The anodic reaction solution contained MDH (24 units per batch), mediator, and buffer. The solution was deoxygenated before insertion into the fuel cell, which was performed with the peristaltic pump, trying to avoid gas bubbles and contact with the air. Acid production was clearly observed in the stack test. In three hours the pH of the anolyte had decreased to pH 6. Adjustment of the pH was difficult because of the small volume and because the enzyme was not immobilised. Therefore no anolyte could be removed before adding alkali. Consequently, strong alkali had to be added and most probably some of the enzyme experienced pH shock and may have been denatured. When the stack was dismantled there was light-greyish precipitate inside the anode. This was either the precipitate from inactivated mediator which turns light brown when oligomerised, coagulated enzyme, or a mixture of the two. Clogs in anodic pipes were also observed.

The OCV of the DMBFC unit cell was regularly 1.2-1.4 V. Figure 5.10 shows the OCV of the individual cells of the stack of one experiment. Only two of them are close to that of a DMBFC unit cell. The OCVs of the unit cells ranged from 1.2-1.35 V to 0.7-0.8 V from one experiment to another. The only difference is that in the stack the enzyme was in solution and not fixed in the anode current collector, as in the unit cell. In the case shown above, the OCV of the unit cells #4 and #8 was especially low at the beginning. The circulation of cathode electrolyte had a positive effect for a moment. Nevertheless, a short circuit is clearly observable; the OCV drops by 200-500 mV in less than 24 hours. The likely cause is leakage between the anode and cathode chambers. In Figure 5.11 the results of a fairly good test are shown.

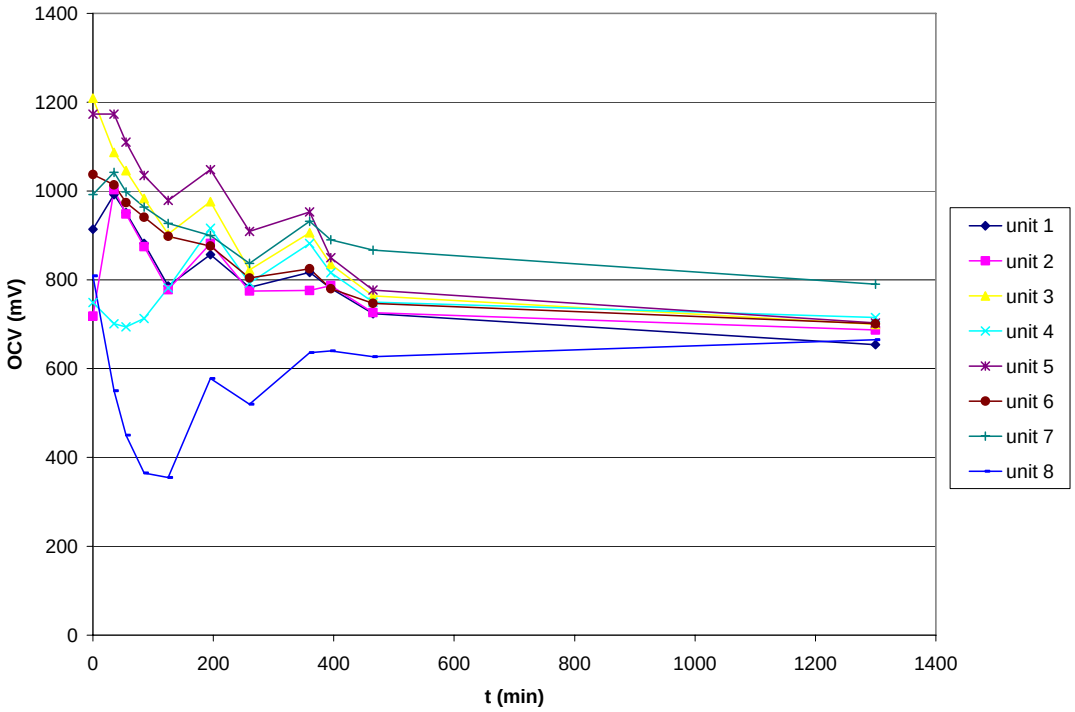
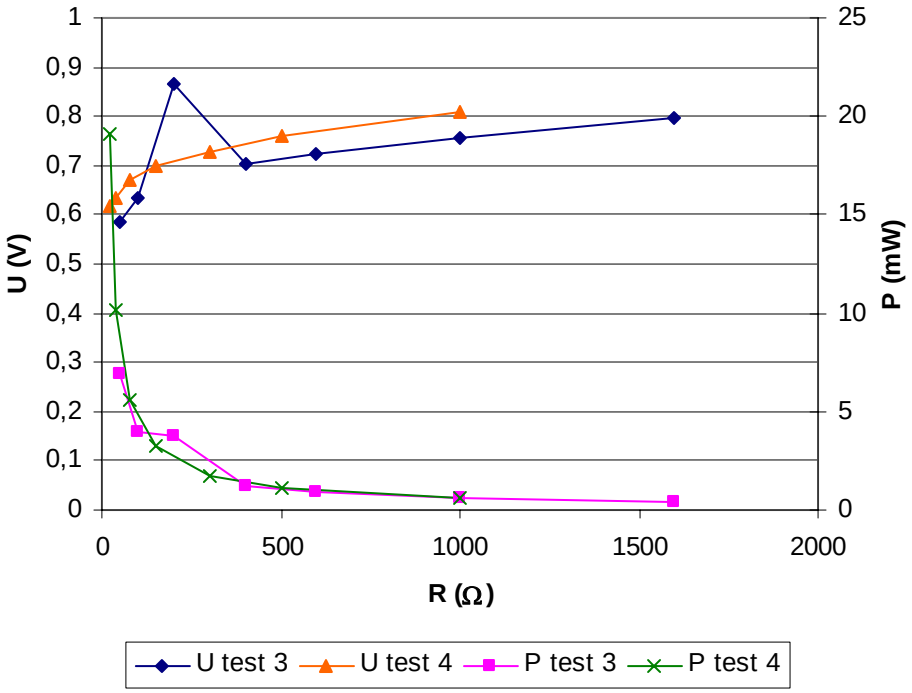
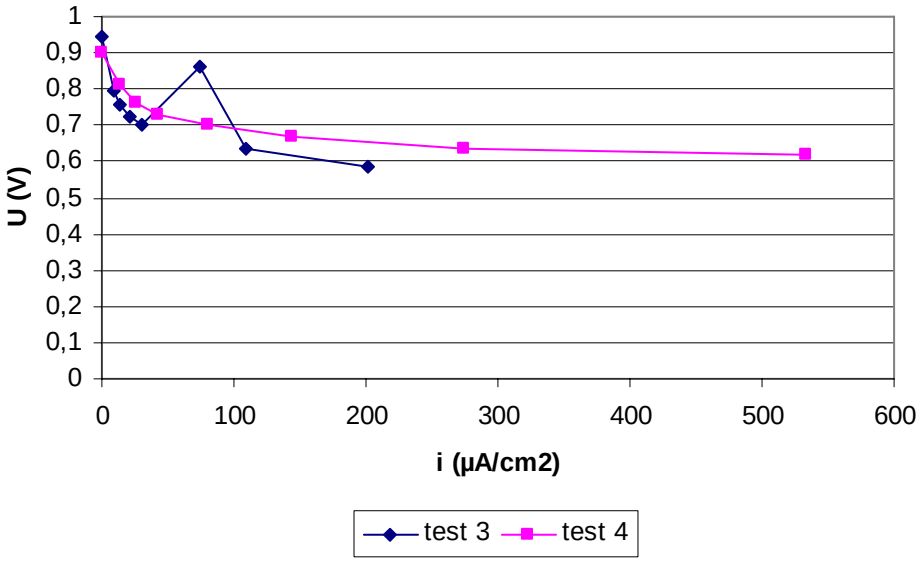


Figure 5.10. Behaviour of open circuit voltage of the stack; individual cells are shown separately. The cathode solution was circulated at the time points 195 min and 360 min.



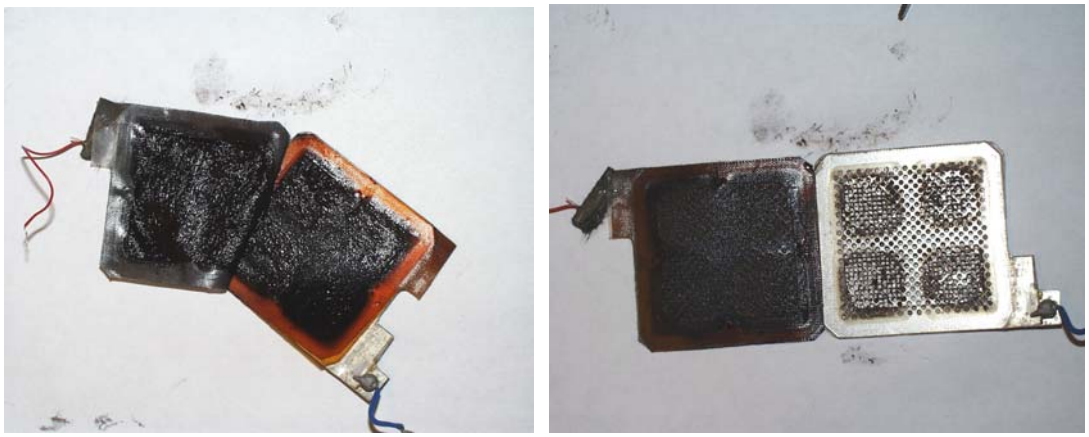
a.



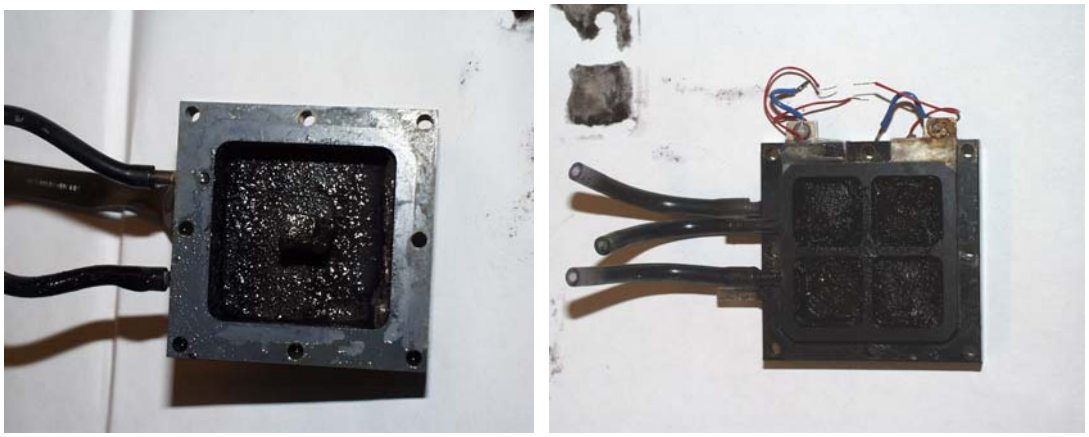
b.

Figure 5.11. a. Voltage and power of the stack as function of resistance; b. dependence of current density of the voltage.

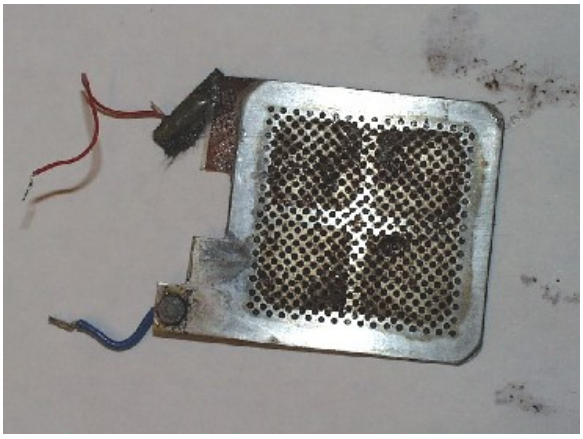
Figure 5.12 illustrates the condition of individual components after the experiment. The mud-like precipitate produced at the cathode is quite clearly seen in Figure 5.12c, which shows the cathode tank (and also the cathodic end of the stack). As well as in Figure 5.12d the cathodic current collector is covered with the precipitate. On the anodic current collector (Figure 5.12e), precipitate is also observed; its origin is most probably oligomerised TMPD, which has brownish colour.



a. b.



c. d.



e.

Figure 5.12. Photos of a dismantled DMBFC stack of 8 unit cells: a. Cathode side of the membrane, with the cathode current collector folded to the left. The membrane (brownish sides seen in the photo) is on the stainless steel plate; b. The anode current collector on the right and the membrane on the left; c. The cathode tank (i.e., the first cathode chamber), the protruding beam (in the centre) is the forcing pipe of the membrane pump. Note the mud-like precipitate covering the surfaces; d. Spacer and membrane support from the cathode side; e. The anode current collector from the side in contact with the anolyte. Part of the membrane can be seen in the top left-hand corner.

5.7 Overall assessment of DMBFC performance

The parameters affecting the performance of the DMBFC are the activity of the enzyme and its concentration, the stability of the mediator, and a large enough potential difference between the anode and cathode.

Figure 5.13 below illustrates the development of enzymatic fuel cell studies utilising methanol and MDH since the very beginning. The results were achieved with the unit cell structure. The improvement is mostly due to fixing the enzyme on a carrier material and a more efficient cathode. In the first year, 2000, the enzyme was free in solution and an oxygen diffusion cathode was applied.

In 2001-2002 the enzyme was fixed; several methods were experimented with. Finally, the graphite paste proved to be best solution for MDH. Fixing the enzyme together with mediator is likely to enhance electron transfer as a result of the smaller distance between the active molecules and the electrode compared to electron transfer by diffusion. Additionally, the longer operating time of the system (7-14 days versus 2-3 days) indicated improved enzyme lifetime.

In 2003 the closed cathode was implemented in order to eliminate the observed limitation of the reaction at the cathode. The OCV value was increased from the early 0.4 V to 1.4 V. The best performance values obtained were 10 mW (625 $\mu W/cm^2$) at a 100 Ω load for 4-5 hours with 2 units of MDH (Table 5.3). The rate of fuel utilisation was observed to be approximately 50%. During the research a low enzyme concentration was utilised on purpose. An improvement of current density requires a higher enzyme load at the anode.

Figure 5.14 shows the biofuel cells developed in the research. The first test cell was utilized in the microbial fuel cell study and measured 9.5 cm × 5 cm × 13 cm (width × depth × height). The second generation of test cells (not shown) was thinner with depth of 3 cm. After a few development steps the DMBFC unit cell measured 6 cm × 4 cm × 5 cm.

The internal resistance R_{IN} contains the activation resistance R_A and the ohmic resistance R_Ω. The activation resistance is a product of the chemistry in the cell, the kinetics of the reaction and the electron transfer. In a biofuel cell activation resistance forms an important part of the total internal resistance. Ohmic resistance is related to the conductivity of materials. The internal resistance was a couple of hundred ohms in 2000. At that time the enzyme and the mediator were in solution. In 2001 the internal resistance was reduced to approximately 100 Ω and in 2002 to around 50 Ω. Fixing the enzyme into the paste resulted in a lower internal resistance value. The introduction of a closed cathode reduced it further to 20 Ω or less.

The pH gradient between the anode and the cathode is significant (pH 10 versus pH 2). The potential difference of the electrodes is large enough to ensure the operation. However, the gradient is one factor affecting the internal resistance; the protons have to move towards higher concentration. Nevertheless, a backflow of protons from the cathode to the anode was not observed.

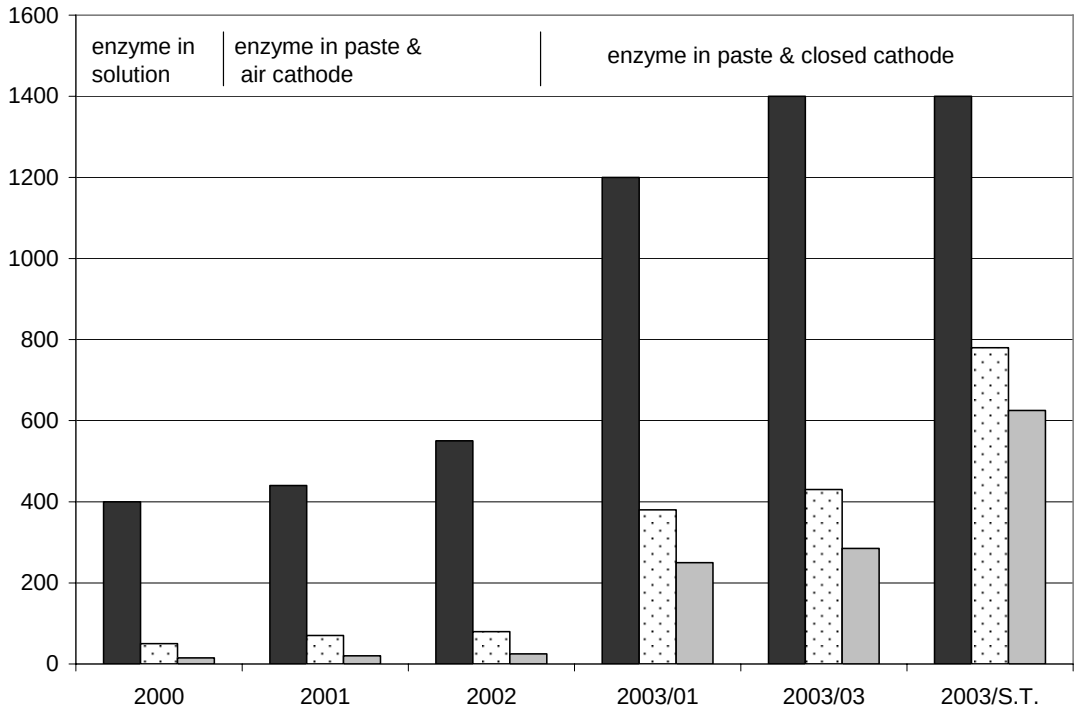
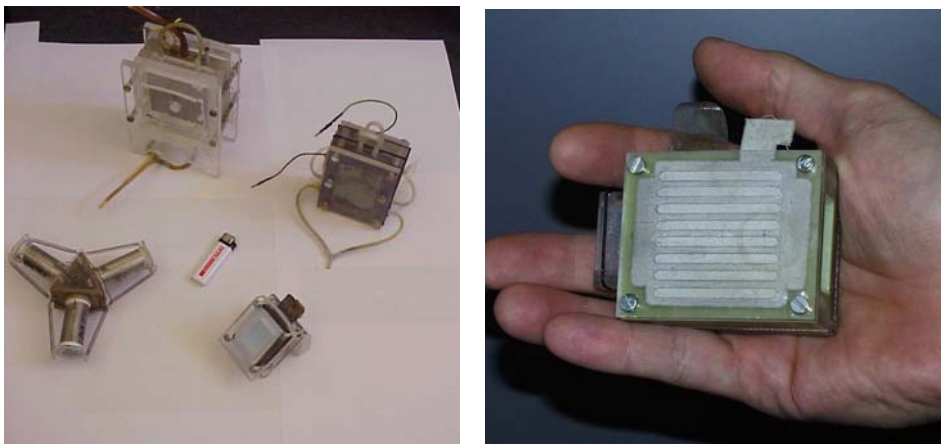


Figure 5.13. Progress of the DMBFC performance during the years 2000-2003; S.T. = short-term. Data obtained at optimal power output condition, c(MDH) = 2 U. (black = open circuit voltage (mV), dotted = current density (μA/cm^2), grey = power density (μW/cm^2)).



a. *b.*

Figure 5.14. Biocatalyst fuel cells developed at the Automation Technology Laboratory: a. The first enzymatic fuel cell at top right (9.5 cm × 5 cm × 13 cm), the small one at down right was modified from a biofuel cell purchased from NCBE (UK), the other two were product of the preceding microbial fuel cell study; b. DMBFC unit cell (6 cm × 4 cm × 5 cm).

Table 5.3. Characteristics of the developed DMBFC with an oxygen diffusion cathode and closed cathode. Power output is the average power during the experiment period. c(MDH) = 2 U.

Parameter	O2 diffusion cathode (2002)	Closed cathode (2003/03, long-term)	Closed cathode (short-term)
OCV	500 mV	1400 mV	1400 mV
Short circuit current	20 mA	50 mA	-
Power	0.5 mW @ ~100 Ω (36 µW/cm2)	4 mW @ ~100 Ω (285 µW/cm2)	10 mW @ ~100 Ω (625 µW/cm2)
Current	2 mA (140 µA/cm2)	6 mA (430 µA/cm2)	12 mA (780 µA/cm2)
Capacity	700 mAh	2000 mAh	-
Internal resistance	< 50 Ω	< 20 Ω	< 20 Ω
Operating time	2 weeks	2 weeks	4-5 h

Stable pH is essential both at the anode and at the cathode, but it is especially important at the anode because of the formic acid produced in the enzymatic reaction. The enzyme activity is lost if the pH drops too low. The production of formic acid poses a significant technological challenge for the development of the DMBFC – it is a fundamental limitation. The MDH enzyme has proven to be robust and viable, but the pH has to be kept around pH 9. For short-term experiments one can use buffer solutions as described here. For a consumer product the capacity of the buffer solutions is not adequate.

The formic acid has to be eliminated with a more efficient method. Novel materials such as active membranes may provide a solution to the problem of acid production, for example a controlled diffusion of alkali to the anode chamber. Another method is to utilise a second enzyme, such as formate dehydrogenase (FDH), to oxidise the formic acid to CO_2 and water. This was in fact tested but the optimum conditions of these two enzymes are too far apart. The optimal pH of FDH is 7. However, the combination of two enzymes is a viable solution if the optimal conditions are close to each other, as is the case with alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (AldDH), for example (Akers et al., 2005). This combination oxidises ethanol to acetic acid through acetic aldehyde.

In principle, TMPD is a good mediator for this case; it has potential close to that of PQQ of MDH and the distinctive colour change between redox states is practical. One can see the state of the reaction at a glance if the cover material is transparent. The downside is the oligomerisation, which was accelerated in fuel cell conditions. The performance of the DMBFC was observed to increase with addition of 0.2%w/w of a metal oxide. The best results were obtained with titanium oxide. The likely cause is the stabilisation of the mediator by the added metal oxide; similar observations have been reported in the literature (Peihong et al., 1995).

In the simulated fuel cell conditions the half-life of TMPD was decreased to 4 days from the 10 days determined for the spontaneous reaction. Additionally, the mediator was observed to diffuse from the graphite paste presently utilised. A proper immobilisation method for TMPD would improve the performance of the

DMBFC. TMPD can also act as a capacitor. A unit cell loaded with 5 mM TMPD solution generated 5 $\mu W/cm^2$, whereas an identical cell generated 7.5 $\mu W/cm^2$ in the presence of MDH (0.5 units/cell). The effect of MDH is clearer if one compares the capacity; it was 3.3 times greater in the presence of MDH (2 mAh vs. 0.6 mAh at 10 kΩ).

The target power output for the DMBFC stack was 100 mW (1.7 mW/cm^2) at 0.5-0.7 V and OCV 1.2-1.4 V. The power output achieved was approximately 0.17 mW/cm^2 and OCV 0.7-1.2V. The stack suffered from mechanical problems such as leakage and clogged channels. Further study is required to develop an improved design and to solve the internal circulation of fluids. In addition, MDH would preferably be immobilised in order to increase the performance and facilitate the pH control of the anolyte. For a practical power source the walls were also too thick. The corrosive catholyte determined the material thickness.

In the preceding microbial fuel cell study the biological system was observed to have a self-regulating feature (Zhang, 1995; Zhang and Halme, 1997). Self-regulation means that the fuel was not consumed if current was not drawn from the fuel cell. This self-regulation is based on microbes being self-supporting organisms which continue metabolic processes in any event. The existence of a similar characteristic in the DMBFC has not been verified. Nevertheless, there is a strong supposition that the enzymatic reaction would have some sort of feedback regulation. The question is whether the enzymatic reaction will go on as long as there is substrate (fuel) available or whether the reaction will halt due to reaching some kind of an equilibrium state. The feedback regulation is believed to function in such a way that the enzyme will oxidise substrate molecules as long as there are free mediators ready to capture the electrons from the active centre. When every mediator molecule has become oxidised, the enzyme cannot release the electrons from the active site and the enzymatic reaction is halted until some of the mediators are reduced at the electrode. This hypothesis remains to be verified in future study.

The novel protocol of loading each unit cell of the fuel cell system reported in Kielosto (2005) was shown to have a statistically positive effect on the performance of the DMBFC. The principle is to periodically load individual cells connected in parallel. Each cell will have a rest period and if one of the cells fails, the control system could discard it. The cause of the positive effect is most probably the refreshment of the immediate surroundings of the electrode. The repeated cycles of switching the load on and off are assumed to cause the movement of charged particles near the electrode. Further study with higher enzyme loads is necessary in order to verify the overall benefit. The periodical loading principle is believed to be also applicable to regular fuel cell technology.

The sophisticated method of immobilisation mentioned is probably the main reason for the higher performance values of the Akermin group. They have reported in a scientific journal 1.6-2 mW/cm^2 for an ADH-AldDH$|O_2$ biofuel cell running on ethanol and as high as 5 mW/cm^2 in a conference forum (Akers et al., 2005; Akers, 2005). In both cases the enzyme was immobilised in modified Nafion. The first system was a U-shaped reactor with anodic and cathodic volumes of 50 ml and had a standard platinum electrode as its cathode. The second system was claimed to have bilirubin oxidase as a cathodic catalyst but no specific details of the system

were disclosed. If one compares the lower figures to those reported here, the difference is 6-7 times greater as regards long-term performance and 2-3 times greater as regards short-term performance. Besides the immobilisation method, the Akermin group has the advantage of a bigger electrode volume, 50 ml versus the 15-20 ml of the DMBFC. This is an essential factor, as greater volume means a larger buffering capacity. On the other hand, according to their publication the system was clearly a laboratory test device. As for the DMBFC, the system design has been taken one generation closer to a practical power source.

Chapter 6

SUMMARY

A novel type of biofuel cell was developed in the study. The concept of a direct methanol biofuel cell, or DMBFC, consists of an early prototype of a power source for low-power electronic appliances. The operating principle is the enzymatic oxidation of methanol with methanol dehydrogenase in the anode chamber and mediated electron transfer by TMPD. Two kinds of cathodes were utilised: a semi-commercial oxygen diffusion cathode and a potassium permanganate cathode, i.e., a closed cathode.

The best performance was achieved with the DMBFC unit cell equipped with a closed cathode. The basic characteristics were also determined using a unit cell. The OCV achieved with a closed cathode was 1.4 V. Maximum power output was 625 $\mu W/cm^2$ for 4-5 hours; longer-term performance was 285 $\mu W/cm^2$; the internal resistance was below 20 Ω. The power density of the DMBFC improved 19-fold during the 3-year study (285 $\mu W/cm^2$ in 2003 versus 15 $\mu W/cm^2$ in 2000). Additionally, the development of the composition of the cells reduced the internal resistance from 200-300 Ω to approximately 20 Ω. The stability of the mediator was improved by adding a metal oxide to the anodic paste. The most important issue for future research is to solve the problem of the neutralisation of the acid produced in the anode. The utilisation of a buffer is a practical solution for short-term laboratory runs, but for the longer term another method has to be invented. The results of the development were applied to design a stack-structured biofuel cell.

Important characteristics of biofuel cells, besides power and current density, include open circuit voltage and internal resistance. The open circuit voltage in a biofuel cell based on mediated electron transfer is the potential difference between the mediator and the cathode. Equally important is the internal resistance, which is the sum of the activation and ohmic resistance. It is typical of fuel cells to have a gap between the open circuit potential and the operational potential; the drop is caused by the reaction slowness and material resistance. The voltage drop is larger in biofuel cells because of slower reaction kinetics.

Fixing the enzyme and the mediator and introducing an effective cathode lowered the internal resistance substantially; in this case study, the reduction was approximately 90% of the value at the beginning of the study. In this case the enzyme and mediator were fixed using a simple but functional method. A more sophisticated method of immobilising the enzyme and mediator, such as enzyme wiring or enclosing the enzyme inside a conductive polymer or polymer web, would enhance electron transfer. The latter method would also protect the enzyme from environmental stress. If an appropriate contact between the enzyme and electron conducting material is created, the mediator may become unnecessary. Thus one step in the electron transfer would be eliminated, as would issues of mediator stability. The application of conducting polymer would make possible simple electrode structures in which the catalyst layer is of the same material as the current collector on the electrode surface. Additionally, incorporation of the

biocatalyst into a polymer-based electrode or membrane would enable the design of a biofuel cell with a MEA, which is familiar from metal catalyst fuel cells.

As to the cathode of the DMBFC, the utilisation of a strong oxidant such as potassium permanganate is a good solution for laboratory testing, but not practical for a consumer device. The corrosiveness of the oxidant requires perfect sealing and it wears out the structural materials. In this case the used oxygen diffusion cathode used performed poorly, but in principle it also has advantages: the oxidant would never run out. The closed cathode concept includes the necessity of changing the oxidant. A practical solution for a biofuel cell with a closed cathode requires the combined refreshment of the anodic fuel and the oxidant.

The novel protocol of loading each unit cell of a fuel cell system was shown to have a statistically positive effect on the performance of the DMBFC. The cause is most probably the refreshment of the immediate surroundings of the electrode. The periodical loading principle is believed to be applicable to regular fuel cell technology.

At the moment the commercialisation of mobile fuel cells seems to be more realistic than it was a couple of years ago. The legislation and regulations considering the transport of fuel cartridges and fuel cells will take effect in 2007. In addition, cooperation between the foremost fuel cell developers and electronics manufacturers has increased. Yet there is the consumer attitude to overcome, at least in the western world, and the infrastructure of the supply chain of fuel cartridges to create. However, the market pull created by the power eaters, such as intelligent mobile phones, is strong and works in favour of the fuel cell technology. If one compares metal catalyst fuel cells and biofuel cells, the performance gap is evident. Nevertheless, niche applications for biofuel cells do exist, such as blood sugar level monitoring. It is more challenging to replace batteries with biofuel cells. Still, it is reasonable to expect that advances in biotechnology and material science will create solutions to improve the power and current density of biofuel cells, thus widening the application area from the niche markets already described.

Themes of future research in respect of the DMBFC include improvement of pH control, further reduction of the internal resistance, verification of the benefit of the periodical loading protocol, and optimisation of the mechanical structure of the stack. As to research in the field of the biofuel cells in general, the credibility gap would be eliminated if a closer imitation of the metabolic pathways inside cells could be created. One conceptual approach in this direction utilising possibilities offered by micromechanical engineering was found in the literature (Kjeang et al., 2006).

LIST OF REFERENCES

Akers, N., 2005. Biofuel cell potential for application in portable devices. In: Conference handouts of the Small Fuel Cells 2005 – The Workshop. Washington DC, USA, April 27-29, 2005. Knowledge Foundation.

Akers, N.L., Moore, C.M., and Minteer, S.D., 2005. Development of alcohol/O_2 biofuel cell using salt-extracted tetrabutylammonium bromide/Nafion membranes to immobilize dehydrogenase enzymes. Electrochimica Acta 50, 2521-2525.

Allen, R.M. and Bennetto, H.P., 1993. Microbial fuel cells: electricity production from carbohydrates. Applied Biochemistry and Biotechnology 39, 27-39.

Anthony, C., Ghosh, M, and Blake, C.C.F., 1994. The structure and function of methanol dehydrogenase and related quinoproteins containing pyrrolo-quinoline quinine. Biochemical Journal 304, 665-674.

Anthony, C., 1996. Quinoprotein-catalysed reactions. Biochemical Journal 320, 697-711.

Anthony, C., 2004. The quinoprotein dehydrogenases for methanol and glucose. Archives of Biochemistry and Biophysics 428, 2-9.

Baker, A., Jollie, D, and Adamson, K.-A., 2005. Fuel Cell Today market survey: Portable applications. published 28 September 2005 Available: http://www.fuelcelltoday.com/FuelCellToday/IndustryInformation/IndustryInformationExternal/Reports/DisplayReport/0,1620,1034,00.html?Category=Survey. Cited 16 March 2006.

Bazhang, Y., Long, N., Moussy, Y., and Moussy, F., 2006. A long-term flexible minimally-invasive implantable glucose biosensor based on an epoxy-enhanced polyurethane membrane. Biosensors and Bioelectronics 21, 2275-2282.

Bennetto, H.P., 1984. Microbial fuel cells. Life Chemistry Reports 2, 363-453.

Bossel, U., 2000. The birth of the fuel cell. Offset- und Dissertations-Druck Jürgen Kinzel, Göttingen, Germany: European Fuel Cell forum, 157 p.

Bostaph, j., Xie, C., Pavio, J., Fisher, A.M., Mylan B., and Hallmark, J., 2002. 1 W direct methanol fuel cell system as a desktop charger, http://www.motorola.com/mot/doc/0/130_MotDoc.pdf, published 12.8.2002. Cited 27.5.2004.

Bullen, R.A., Arnot, T.C., Lakeman, J.B. and Walsh, F.C., 2006. Biofuel cells and their development. Biosensors and Bioelectronics 21, 2015-2045.

Chang, H., 2003. Technical and commercial issues of DMFC: 5 W for mobile device and 100 W for portable power, Samsung AIT. In: Conference handouts of the Small Fuel Cells 2003 Conference. New Orleans, USA, May 7-9, 2003. Knowledge Foundation.

Davis, F. and Higson, S.P.J., 2006. Biofuel cells – Recent advances and applications. Biosensors and Bioelectronics. Available online 16 June 2006. http://www.sciencedirect.com/science?_ob=MImg&_imagekey=B6TFC-4K6CPMS-1-F&_cdi=5223&_user=952938&_orig=search&_coverDate=06%2F16%2F2006&_sk=999999999&view=c&_alid=455168089&_rdoc=1&wchp=dGLbVtz-zSkzk&md5=c6bd0e5d7cf9c21c5d981d3c84e8f6da&ie=/sdarticle.pdf. Cited July 2006.

Day, D.J. and Anthony, C., 1990. Spectrophotometric assay of Methanol dehydrogenase. Methods in Enzymology 188, 210-216.

Dumé, B., 2006. Small fuel cells, big opportunities. Fuel Cell Review 2 (6), 27-28.

Frank, J., Dijkstra, M., Duine, J.A., and Balny C., 1988. Kinetic and spectral studies on the redox forms of methanol dehydrogenase from *Hyphomicrobium X*, European Journal of Biochemistry 174, 331-338.

Fuel Cell Review, 2005. Another take on portability, Fuel Cell Review 2 (3), 30-31.

Fuel Cell Today, 2005. Micro Methanol Fuel Cells Get Approval for Airplane Use. Published November, 08 2005. Available on-line http://www.polyfuel.com/pressroom/News/11-8-05%20micro-methanol-fuel-cells-get-approval.pdf#search=%22ICA0%20methanol%22. Cited 23 August 2006.

Gottesfeld, S., 2003. Direct methanol fuel cells for portable power applications. In: Conference handouts of the Small Fuel Cells 2003 Conference. New Orleans, USA May 7-9, 2003. Knowledge Foundation.

Gottesfeld, S., 2004. DMFCs power up for portable devices. Fuel Cell Review 1 (2), 25-29.

Guo, Z. and Cao, Y., 2004. A passive fuel delivery system for portable direct methanol fuel cells. Journal of Power Sources 132, 86-91.

Halme A., Zhang, X.-C., Rintala, N., 1998. Monitoring and Control of a Bacteria Fuel Cell Process by Colour Analysis, In: Proceedings of the 7th international Conference on Computer Applications on Biotechnology. May 31 - June 4, 1998, OSAKA, Japan. 467-472.

Halme, A., Korhola, M., Ranta, A., Suomela, J. and Zhang, X.-C., 2002a. Biocatalytic direct alcohol fuel cell. Patent application 2020893FI, Finland. Submitted June 28, 2002.

Halme, A., Korhola, M., Ranta, A., Suomela, J. and Zhang, X.-C., 2002b. Arrangement and method for producing electric power and the power source. Patent application 2021008FI, Finland. Submitted June 28, 2002.

Heller, A., 1990. Concepts of electrically connecting redox enzymes to metal electrodes. American Biotechnology Laboratory 8 (8) 14-16.

Holladay, J.D., Jones, E.O., Phepls, M., and Hu, J., 2002. Microfuel processor for use in a miniature power supply. Journal of Power Sources 108, 21-27.

IEC Publicly Available Specification 62282-6-1 Microfuel cells safety – Summary of the standard, 2005. Available online: http://www.hydrogenandfuelcellsafety.info /archives/2005/nov/icao_executiveSummary.pdf#search=%22IEC% 2062282-6-1%22. Cited August 23, 2006.

Ikeda, T. and Kano, K., 2001. An electrochemical approach to the studies of biological redox reactions and their applications to biosensors, bioreactors, and biofuel cells. Journal of Bioscience and Bioengineering 92, 9-18.

IPCS Health and safety guide no. 105: Methanol (1997), Available on-line: http://www.inchem.org/documents/hsg/hsg/v105hsg.htm#PartNumber:2IP. Cited 30 August 2006.

Jane's, 2006. Powerful Challenges – A view on the evolving state of powering technologies for soldier-borne equipment, available on-line: http://www.mtimicrofuelcells.com/pdf/20050425_Janes_White_Paper-Powerful_Challenges.pdf. Cited August 23, 2006.

Kangasniemi, T., 2002. Polttokennoteholähteen elektroniikkaosa, Bachelor's Thesis, EVTEK ammattikorkeakoulu, Vantaa, Finland 2002.

Kano, K., and Ikeda, T., 2000. Fundamentals and practices of mediated bioelectrocatalysis. Analytical Sciences 16, 1013-1021.

Katakis, I. and Heller, A., 1997. Electron transfer via redox hydrogels between electrodes and enzymes. In: Frontiers in Biosensorics I. Eds. Scheller, F.W., Schubert, F., and Fedrowitz, J.. Switzerland: Birkhäuser Verlag Bset, 231-241.

Katz, E., Willner, I., and Kotlyar, A.B., 1999. A non-compartmentalized glucose$|O_2$ biofuel cell by bioengineered electrode surfaces. Journal of Electroanalytical Chemistry 479, 64-68.

Kazama, T., 2004. Fuel Cells: Business Opportunities and Issues to be Resolved. NRI Papers No. 80. Published September 1, 2004. Available online: http://www.nri.co.jp/english/opinion/papers/2004/pdf/np200480.pdf, Cited January 25, 2005 .

Kelly, I., 2003. Design of a robotic predator: The SlugBot. Robotica 21, 399-406.

Kelty, K., 2003. Small Fuel Cells vs Li-ion Battery: A Battery Manufacturers View, In: Conference handouts of the Small Fuel Cells Conference 2003, May 7-9 2003, New Orleans, USA. Knowledge Foundation.

Kielosto, S., 2005. Effect of loading waveform to the power output of an enzymatic closed-cathode biofuel cell. Master's Thesis, Helsinki University of Technology, Automation Technology Laboratory, Espoo, 69 p.

Kjeang, E., Sinton, D., and Harrington, D.A., 2006. Strategic enzyme patterning for microfluidic biofuel cells. Journal of Power Sources 158, 1-12.

Kotilainen, S., 2006. Samsung kokeilee polttokennoja kännyköissä, Tietokone. Published May 22, 2006. Available on-line http://www.tietokone.fi/uutta/uutinen.asp?news_id=27102&tyyppi=1; Cited 23 May 2006.

Larminie, J. and Dicks, A., 2001. Fuel cell systems explained. Great Britain: John Wiley & Sons, 308 p.

Larsen, R., Ha, S., Zakzeski, J., and Masel, R.I., 2006. Unusually active palladium-based catalysts for the electrooxidation of formic acid. Journal of Power sources 157, 78-84.

Liberatore, M.J., Hozer, L., Sreeram, A.N., Kumar, R., Bindra, C., Fan, Z., 2002. Enzyme battery. Patent Application 200220001739 USA, Published January 3, 2002.

Logan, B.E., Hamelers, B., Rozendal, R., Schöder, U., Keller, J., Freguia, S., Aelterman, P., Versraete, W., and Rabaey, K., 2006. Microbial fuel cells: Methodology and technology. Environmental Science & Technology 40, 5181-5192.

Mano, N., Mao, F, and Heller, A., 2002. A Miniature Biofuel Cell Operating in A Physiological Buffer. Journal of American Chemical Society 124, 12962-12963.

Mano, N., Mao, F., Heller, A., 2003a. Characteristics of a miniature compartment-less glucose-O_2 biofuel cell and its operation in a living plant. Journal of American Chemical Society 125, 6588-6594.

Mano, N., Mao, F, Shin, W., Chen, T., and Heller, A., 2003b. A Miniature Biofuel Cell Operating at 0.78 V, Chemical Communications, 518-519.

Mano, N., Mao, F, and Heller, A., 2004. A Miniature Membrane-less Biofuel Cell Operating at +0.60 V under physiological conditions. ChemBioChem 5, 1703-1705.

Matsuhita, k., Toyama, H., Yamada, M., and Adachi, O., 2002. Quinoproteins: structure, function, and biotechnological applications. Applied Microbiology and Biotechnology 58, 13-22.

McNamee, G.C. and Acker, W.P., 2003. Passively pumped liquid feed fuel cell system. Patent number US2003/0095872 A1 USA. Published May 22, 2003. Filed November 19, 2002.

Michaelis, L., Schubert, M.P., and Granick, S., 1939. The free radicals of the type of Wurster's salts. Journal of American Chemical Society 61, 1981-1992.

Moore, C.M., Akers, N.L., Hill, A.D., Johnson, Z.C., and Minteer, S.D., 2004. Improving the environment for immobilized dehydrogenase enzymes by modifying Nafion with tetraalkylammonium bromides. Biomacromolecules 5, 1241-1247.

Mukoma, P., Jooste, B.R., and Vosloo, H.C.M, 2004. Synthesis and characterisation of cross-linked chitosan membranes for application as alternative proton exchance membrane materials in fuel cells. Journal of Power sources 136, 16-23.

Nano Battery, 2005. Exceptional PolyFuel membrane durability enables over 5000-hour portable fuel cell lifetimes; crucial customer requirement well surpassed as hydrocarbon technology keeps on going. Published October 31, 2005. Available on-line: http:// www.voyle.net/Nano%20Battery%20 Nano%20Battery%202005-0020.htm. Cited August 29, 2006.

Palmore, G.T.R., Bertschy, H., Bergens, S.H., and Whitesides, G.M., 1998. A methanol/dioxygen biofuel cell that uses NAD^+-dependent dehydrogenases as catalysts: application of an electro-enzymatic method to regenerate nicotinamide adenine dinucleotide at low overpotentials. Journal of Electroanalalytical Chemistry 443, 155-161.

Palmore, G.T.R. and Kim, H.-H., 1999. Electro-enzymatic reduction of dioxygen to water in the cathode compartment of a biofuel cell. Journal of Electroanalalytical Chemistry 464, 110-117.

Palmore, G.T.R. and Whitesides, G.M., 1994. Microbial and Enzymatic fuel cells. ACS Symposium Series 566 (1), 271-290.

Pattekar, A.V. and Kothare. M.V., 2004. A microreactor for hydrogen production in micro fuel cell applications. Journal of Microelectromechanical Systems 13, 7-18

Peihong, C., Fryling, M.A., McCreery, R.L., 1995. Electron transfer kinetics at modified carbon electrode surfaces: the role of specific surface sites. Analytical Chemistry 67, 3115-3122.

Plotkin, E.V., Higgins, I.J., and Hill, H.A.O., 1981. Methanol dehydrogenase bioelectrochemical and alcohol detector. Biotechnology Letters 3 (4), 187-192.

Potter, M.C., 1912. Electrical effects accompanying the decomposition of organic compounds. Proceedings of Royal Society B 84, 260-276.

Ranta, A., Zhang, X., and Halme, A., 2003. Conceptual prototype of a direct methanol biocatalytic fuel cell, A poster presentation at the Small Fuel Cells 2003 conference. New Orleans, USA, May 8-9, 2003. Knowledge Foundation.

Ren, X., Gottesfeld, S., Becerra, J.J., and Hirch, R.S., 2004. Fluid management component for use in a fuel cell. Patent number US2004/0062980 A1 USA, Publication date April 1, 2004. Filed September 30, 2002.

Rice, C., Ha, S., Masel, R.I., Waszczuk, P., Wieckovski, A., and Barnard, T., 2002. Direct formic acid fuel cells. Journal of Power Sources 111, 83-89.

Schuhmann, W., 2002. Amperometric enzyme biosensors based on optimised electron-transfer pathways and non-manual immobilisation procedures. Journal of Biotechnology 82, 425-441.

Senda, M., 1990. Biocatalyst electrodes and their applications to sensors and reactors, In: Enzyme engineering, eds. Okada, H., Tanaka, A., Blanch, H.W., Annals of New York Academy of Sciences Vol. 613. New York, USA: The New York Academy of Sciences. 79-94.

Soukrahev, V., Mano, N., and Heller, A., 2004. A four-electron O_2-electroreduction biocatalyst superior to platinum and a biofuel cell operating at 0.88 V. Journal of American Chemical Society 126, 8368-8369.

Stanley, K., Wu. J., and Parameswaran, M., 2005. Cathode effects for microfuel cells. In: Conference handouts of the Small Fuel Cells Conference 2005, April 27-29, 2005. Washington DC, USA. Knowledge Foundation.

Tarasevich, M.R., 1985. Bioelectrocatalysis. In: Comprehensive treaties of electrochemistry. Eds. Srinivasan, S., Chizmadzhev, Y.A., Bockris, J.O'M, Conway, B.E., and Yeager, E.. New York, USA: Plenum Press. 231-295.

UTC 2006. PureCell™ Power Solution. Available on-line http://www.utcfuelcells.com/fs/com/bin/fs_com_Page/0,9235,03100,00.html. Cited August 23, 2006.

Volfová, O., 1980. Effect of formate on methanol oxidizing enzymes. Biotechnology Letters 2 (8), 369-374.

Wilkinson, S. and Campbell C., 1996. "Green" bug robots – renewable environmental power for miniature robots. In: Proceedings of 4th IASTED International Conference Robotics and Manufacturing, Paper 247-113, Honolulu, USA, August 19-22, 1996. 275-278.

Wilkinson, S., 2000a. "Gastronome" – A pioneering food powered mobile robot. In: Proceedings of the IASTED (8^{th}) International Conference on Robotics and Applications 2000, August 14-16, 2000, Honolulu, Hawaii, USA, 176-181.

Wilkinson, S., 2000b. "Gastrobots" – Benefits & Challenges of Microbial Fuel Cells in Food Powered Robot Applications. Journal of Autonomous Robots 9 (24), 99-111.

Wilkinson, S., 2001. Hungry for success – future directions in gatrobotics research. Industrial Robot: An International Journal 28 (3), 213-219.

Wilkinson, S., Applegarth, S.P. and Pennington, G.M., 2001a. "Juice from juice"-Electricity generation for carpophagous gastrobots, In: Proceedings of the IASTED International Conference ROBOTICS AND APPLICATIONS. Clearwater (Tampa) Florida, USA. Nov 19-22, 2001. 204-212.

Wilkinson, S., Pennington, G.M, and Applegarth,2001b. Potential role of bees & honey in gastrobot foraging & power generation. In: Proceedings of the IASTED International Conference ROBOTICS AND APPLICATIONS, Clearwater (Tampa) Florida, USA. Nov 19-22, 2001. 213-219.

Xia, Z., Dai., W.-W., Zhang, Y.-F., White, S.A., Boyd, G.D., and Mathews, F.S., 1996. Determination of the gene sequence and the three-dimensional structure at 2.4 Å resolution of methanol dehydrogenase from *Methylophilus* W3A1. Journal of Molecular Biology 259, 480-501.

Yahiro, A.T., Lee, S.M., and Kimble, D.O., 1964. Enzyme utilizing bio-fuel cell studies, Biochimica et Biophysica Acta 88 (2), 375-385.

Zhang, X.-C., 1995. Aspects of modelling and control of bioprocesses – Application of conventional approach and functional state concept. Doctoral Dissertation, Research reports of Automation Technology Laboratory of Helsinki University of Technology, No. 13., Helsinki University of Technology, Espoo, Finland. 75 p.

Zhang, X.-C. , Halme, A., 1997. Effect of size and structure of a bacteria fuel cell on the electricity production and energy conversion rate. Research reports of Automation Technology Laboratory of Helsinki University of Technology, No. 17, Helsinki University of Technology, Espoo, Finland. 10 p.

Zhang, X.-C., Ranta, A., and Halme, A., 2006. Direct methanol biocatalytic fuel cell – Considerations of restraints on electron transfer. Biosensors and Bioelectronics 21, 2052-2057.

Zhang, X.-C., Ylikorpi, T., and Pepe, G., 2005. Biomass-based fuel cells for manned space exploration. European Space Agency Report: Ariadna AO/1-4532/03/NL/MV. 104 p. Available online http://www.esa.int/gsp/ACT/doc/ACT-RPT-04-2301-ARIADNA-Biomass%20based%20fuel%20cells-Helsinki.pdf.

Company web sites

Abbott Diabetes Care, http://abbottdiabetescare.com
Fuel Cell Today: image library, http://www.fuelcelltoday.com/
Hydrocell, http://www.hydrocell.fi
Medis Technologies, http://www.medistechnologies.com/
MTI Microfuel cells, http://www.mtimicrofuelcells.com/
Smart Fuel Cell, http://www.smartfuelcell.de
Toshiba, http://www.toshiba.com
Samsung SAIT, http://www.sait.samsung.com

Appendix 1

SCHEMATIC OF ELECTRONIC CONNECTION BOARD UTILISED IN STACK TESTING