ELECTROCHEMICAL STUDIES OF HYDROLYTIC PRODUCTS FROM REFINED PALM OIL WITH IMMOBILIZED ENZYMES AT MODIFIED CARBON ELECTRODES

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By

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DEDICATION

This work is lovingly and respectfully in honor dedicated to my wonderful husband, Muataz Basheer Aziz for his constant encouragement and motivation, to my beloved parents for their inspiration and to my forever supportive family members.

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LIST OF ABBREVIATIONS

ADH	Alcohol Dehydrogenase
AldDH	Aldehyde Dehydrogenase
^{o} C	Degree Celsius
CVs	Cyclic Voltammograms
DTAB	Dodecyltrimethylammonium bromide
e	Electron
E_{ap}	Applied Potential
E_{pa}	Anodic Peak Potential
E_{pc}	Cathodic Peak Potential
g	Gram
GDH	Glycerol Dehydrogenase
h	Hour
I_{pa}	Anodic Peak Current
I_{pc}	Cathodic Peak Current
j	Current Density

kDa	Kilo Dalton
LSV	Linear Sweep Voltammetry
NADH	β-nicotinamide adenine dinucleotide
PANI	Polyaniline
PMG	Polymethylene green
PoPD	Poly(o-phenylene diamine)
PPy	Polypyrrole
PQQ	Pyrroloquinoline quinine
PTSA	p-toluene sulphonic acid
PVA	Polyvinyl alcohol
P4VP	Poly(4-vinyl pyridine)
SEM	Scanning Electron Microscopy
SLO	Soybean Lipoxygenase
TGs	Triglycerides
V	Volt

KAJIAN ELEKTROKIMIA HASIL HIDROLISIS MINYAK SAWIT TERTAPIS DENGAN ENZIM TERIMOBILKAN PADA ELEKTROD KARBON TERUBAHSUAI

ABSTRAK

Kerja ini menerangkan kesan pH, suhu, masa pengeraman, kepekatan substrat dan berat enzim pada hidrolisis minyak sawit tertapis menggunakan lipase komersial dari yis *Candida rugosa*. keputusan menunjukkan bahawa hidrolisis adalah optimum pada pH 7.5, suhu 37° C, masa eraman 60 minit dan berat enzim dan substrat 0.1 dan 2 g, masing-masing. Asid lemak yang dibebaskan telah digunakan sebagai substrat untuk lipoksigenase yang dipegunkan di permukaan membran Nafion di atas elektrod karbon yang telah diubah suai. Arus yang dijana telah dikaji dengan voltammetri berkitar. Didapati penampan kalium pada pH 7 dan 0.4 mg lipoksigenase adalah penting untuk menghasilkan ketumpatan arus yang lebih tinggi.

Pengelektrooksidaan gliserol (yang diperolehi pada keadaan optimum hidrolisis minyak sawit tertapis, menggunakan lipase komersial) bersama nikotinamida adenina dinukleotida (NAD^+) menggunakan enzim gliserol dehidrogenase yang dipegunkan dalam membran Nafion yang telah dimodifikasi dengan ammonium pada elektrod kain karbon terubahsuai. Disebabkan oleh voltan lampau yang besar yang dihadapi untuk pengoksidaan NADH pada elektrod, lima jenis polimer iaitu polimetilena hijau (PMG), polianilina (PANI), poli(orto-fenilena diamina) (PoPD), poli(4-vinil piridina) (4-VP), dan polipirola (PPy) telah digunakan untuk menjana semula NAD^+ dan mengulang-alik elektron daripada NADH kepada elektrod. Secara amnya, proses redoks gliserol adalah quasi-berbalik dalam julat potensi -0.5 sehingga +0.6 V lawan Ag/AgCl. Kesan pH, kepekatan NAD^+, dan berat lipase telah dikaji untuk mencapai ketumpatan arus yang

lebih tinggi. Pengoksidaan elektrokimia gliserol telah juga dikaji menggunakan dua sistem enzim berlainan, iaitu 1) sistem monoenzim, yang melibatkan alkohol dehidrogenase dipegunkan dalam lapisan Nafion pada permukaan elektrod kain karbon yang diubahsuai dengan PoPD, dan 2) sistem bienzim yang melibatkan alkohol dehidrogenase dan aldehid dehidrogenase yang disekatgerak pada elektrod seperti yang diterangkan tadi. Pembangunan sistem kedua ini bertujuan untuk mengkaji kemungkinan pengoksidaan gliserol berperingkat. Kajian voltammetri ke atas julat potensi -1.5 sehingga +0.1 V berlawan Ag/AgCl, telah menunjukkan bahawa elektrod ini memaparkan, kebanyakannya proses elektrod jenis quasi-berbalik untuk pengoksidaan gliserol. Pelbagai parameter juga telah dikaji untuk mencari keadaan yang optimum. Pada amnya, ketumpatan arus oksidatif maksimum semasa untuk kesemua elektrod adalah pada pH 7 dengan berat lipase 0.1 – 0.15 g dan kepekatan NAD^+ 1 - 3 mM. Penemuan ini dijangka berguna dalam pembentukan katod dan anod dalam sel biofuel menggunakan asid lemak dan gliserol daripada minyak kelapa sawit bertapis.

ELECTROCHEMICAL STUDIES OF HYDROLYTIC PRODUCTS FROM REFINED PALM OIL WITH IMMOBILIZED ENZYMES AT MODIFIED CARBON ELECTRODES

ABSTRACT

This work describes the effect of pH, temperature, incubation time, and substrate and enzyme weights on the hydrolysis of refined palm oil using the commercial lipase from the yeast *Candida rugosa*. It was found that the hydrolysis was optimum at pH 7.5, temperature 37 °C, incubation time 60 min and the enzyme and substrate weights of 0.1 and 2 g, respectively. The released fatty acids were used as a substrate for lipoxygenase immobilized on modified Nafion membrane carbon electrode and the current generated was studied by cyclic voltammetry. Potassium buffer at pH 7 and 0.4 mg of lipoxygenase was found to be crucial in order to produce a higher current density.

The electrooxidation of glycerol (obtained at optimum conditions of hydrolysis of refined palm oil using commercial lipase) in the presence of nicotinamide adenine dinucleotide (NAD^+) using glycerol dehydrogenase enzyme immobilized in ammonium modified Nafion membrane on polymer modified carbon cloth electrodes. Due to the large overvoltage encountered for NADH oxidation at the electrode, five conducting polymers viz. polymethylene green (PMG), polyaniline (PANI), poly(ortho-phenylene diamine) (PoPD), poly(4-vinyl pyridine) (P4VP), and polypyrrole (PPy) were used to regenerate NAD^+ and to shuttle electrons from the NADH to the electrode. In general, the redox processes of glycerol were of quasi-reversible over potential ranges of -0.5 to

+0.6 V *vs* Ag/AgCl. The effect of pH, concentrations of NAD^+, and weight of lipase has been studied to achieve a higher current density. The electrochemical oxidation of glycerol was also studied by another two enzymes system, monoenzyme system, alcohol dehydrogenase immobilized within Nafion layer on the surface of PoPD modified carbon cloth electrode, and a bienzyme system of alcohol dehydrogenase and aldehyde dehydrogenase immobilized similarly on the electrode as described earlier. The latter was aimed for a possible study of multi-step oxidation of glycerol. Voltammetric studies over potential ranges of – 1.5 to + 0.1 V *vs.* Ag/AgCl, have shown that these electrodes displayed mostly quasi-reversible type of electrode processes for the oxidation of glycerol. Various parameters have also been examined for experimental optimum conditions. In general, the maximum oxidative current density for all electrodes was obtained at pH 7 with weight of lipase 0.1- 0.15 g and concentration of NAD^+ 1 - 3 mM. The findings were expected to be useful in the fabrication of cathode and anodes in a biofuel cell using fatty acids and glycerol from refined palm oil.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Palm Oil

In 1434, a Portugese sailor, Gil Eannes first reported about oil palms (*Elaeis guineensis)*. Oil palms are grown mainly in the western part of Africa, Indonesia, Malaysia, and recently in Brazil and Colombia. Oil palm trees grow up to 20 m in height and they grow best at temperature of 24-27^{o}C. Oil palm trees need a humid climate and the cultivated oil palm carry fruit from their fourth year onward and can be harvested for 40-50 years. Malaysia is blessed with good weather conditions which prevail throughout the year make it useful for palm oil cultivation. The first commercial oil palm estate in Malaysia was set up in 1917 at Tennamaran Estate, Selangor. In 1960s, oil palms were commercially cultivated in large scale to avoid over dependence on natural rubber which is major product during earlier years. Since then, palm oil industry has expended fast and has emerged as the most remunerative agricultural commodity, overtaking natural rubber (Norazlan, 2006).

Palm oil is the second most traded vegetable oil crop in the world after soy, Malaysia is among the largest producers and exporters of palm oil in the world, accounting for 52% or 26.3 million tonnes of the total world oils and fats exports in year 2006 (Sumathi *et al.*, 2008). Table 1.1 shows the major palm oil producers, while Table 1.2 details world production of oils and fats.

Table 1.1 World Palm Oil Productions from 1980 to 2008 (Ong *et al.*, 2011)

	Palm oil output / 1000 metric tones						
	1980	**1985**	**1990**	**1995**	**2000**	**2005**	**2008**
Malaysia	2576	4133	6088	8123	10,842	14,962	17,735
Indonesia	691	1243	2413	4220	7050	14,070	19,100
Nigeria	433	386	580	660	740	800	860
Thailand	-	-	232	354	525	685	1160
Colombia	-	-	226	388	524	661	800
Papua New Guinea	-	-	145	223	336	310	400
Cote D'Ivoire	-	-	270	285	278	260	330
Brazil	12	29	66	75	108	160	420
Others	875	1041	1000	5994	5191	1826	2100
World total	4587	6832	11,020	20,322	25,594	33,733	42,904

Table 1.2 World Oil and Fat Production (Ong *et al.*, 2011)

	Volume / 1000 metric tones						
	1980	**1985**	**1990**	**1995**	**2000**	**2005**	**2008**
Palm oil	4543	6832	11,020	20,322	25,594	33,733	40,200
Soyabean oil	13,382	13,974	16,097	15,119	21,743	33,575	35,700
Rapeseed oil	3478	6066	8160	10,936	14,496	16,205	19,900
Sunflower seed oil	5024	6564	7869	7003	9808	9661	10,800
Tallow & grease	6283	6518	6813	7013	8071	8211	8585
Lard	4691	4989	5509	5141	6580	7568	7740
Butter fat	5746	6315	6500	4834	5829	6665	7123
Cottonseed oil	2992	3942	3782	3312	3815	4989	4400
Groundnut oil	2864	3575	3897	4325	4382	4523	4445
Palm kernel oil	571	868	1450	1877	2620	3975	5300
Coconut oil	2716	2627	3387	3253	3147	3257	3500
Olive oil	1701	1796	1855	1863	2513	2916	3000
Others	3695	4430	4552	4618	4995	5100	5840
World total	57,686	68,496	80,891	89,615	113,591	140,378	160,471

1.1.1 Products of Palm Oil

There are two main products produced by the oil palm fruit namely crude palm oil obtained from the mesocarp and crude palm kernel oil obtained from the endosperm (kernel). Figure 1.1 shows a cross section of palm oil fruit. The main by-product and wastes produced from the processing of palm oil are the empty fruit bunches, palm oil mill effluent, sterilizer condensate, palm fibre, and palm kernel shell (Yusoff, 2006).

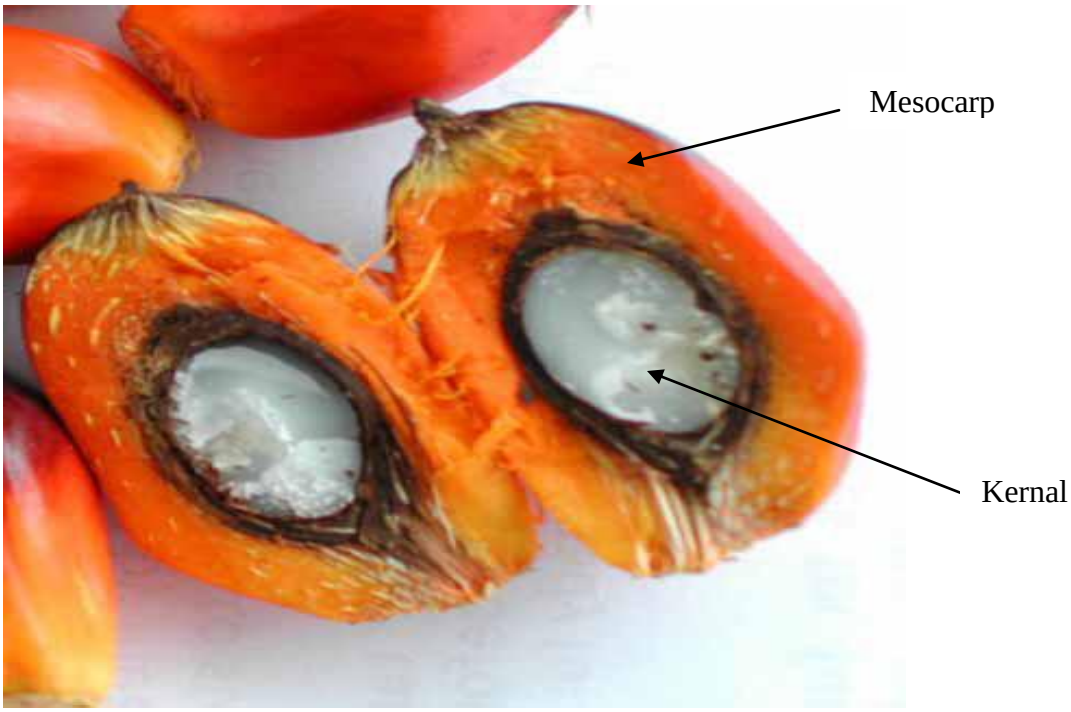


Figure 1.1 Cross section of a fruit.

Mesorcarp accounts for about 60% of the total composition of oil palm fruit. Figure 1.2 shows the composition of mesocarp where the oil accounts for 39% of the overall composition. Crude palm oil is obtained from the mesocarp part of oil palm fruit after undergoing several processes such as sterilization, stripping, extraction, and purification (Norazlan, 2006).

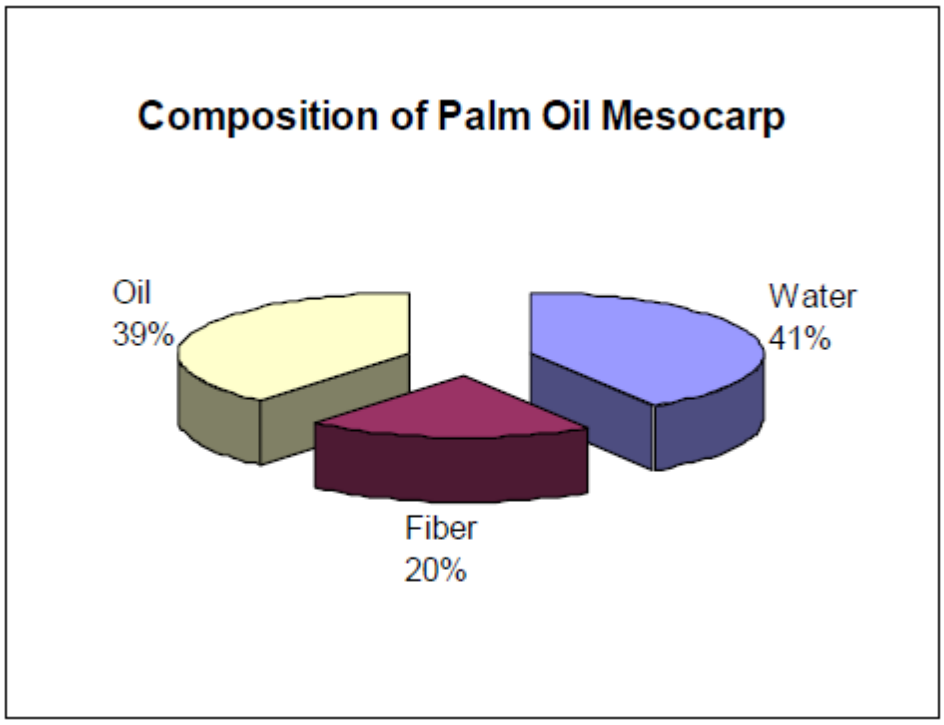


Figure1.2 Composition of palm oil mesocarp (Norazlan, 2006)

1.1.2 Chemistry of Palm Oil

Crude palm oil is semi solid at room temperature; the oil is bright orange-red in colour due to its high content of carotene. Like all oils triglycerides (TGs) are the major constituents of palm oil. Each glycerol molecule is esterified with three fatty acids. The minor components of palm oil are phosphatides, sterols, carotenoids, tocopherols, tocotrienols, and trace metals. The major fatty acids in palm oil are myristic, palmitic, stearic, oleic, and linoleic (Sundram *et al.*, 2003). The fatty acid distribution of the palm oil is given in Table 1.3.

Table 1.3 Fatty Acid Distribution of Palm Oil (Noor *et al.*, 2003)

Name	Fatty acid chain length	% w/w	Formulae	Molecular weight
Lauric	C12:0	0.2	$CH_3(CH_2)_{10}COOH$	200.31
Myristic	C14:0	1.1	$CH_3(CH_2)_{12}COOH$	228.36
Palmitic	C16:0	44.0	$CH_3(CH_2)_{14}COOH$	256.42
Stearic	C18:0	4.5	$CH_3(CH_2)_{16}COOH$	284.47
Oleic	C18:1	39.2	$C_{17}H_{33}COOH$	282.44
Linoleic	C18:2	10.1	$C_{17}H_{31}COOH$	280.43
Others	-	0.9	-	-

Palm oil contains saturated and unsaturated fatty acids in about equal amounts. Most of the fatty acids are present as TGs. There are 7% to 10% of saturated TGs, mainly tripalmitin. The fully unsaturated TGs represent 6 to 12%. The Sn-2 position has specificity for unsaturated fatty acids. Therefore, more than 85% of the unsaturated fatty acids are located in the Sn-2 position of the glycerol molecule. The triacylglycerols in palm oil partially define most of the physical characteristics of the palm oil such as melting point and crystallisation behaviour (Kifli, 1981). Table 1.4 shows the percentage distribution of individual TGs of palm oil.

Table 1.4 Triacyl Glycerol Compositions (%) of Malaysian *Tenera* Palm Oil (Kifli, 1981)

No Double Bond		**1 Double Bond**		**2 Double Bonds**		**3 Double Bonds**		**> 4 Double Bonds**	
MPP	0.29	MOP	0.83	MLP	0.26	MLO	0.14	PLL	1.08
PMP	0.22	MPO	0.15	MOO	0.43	PLO	6.59	OLO	1.71
PPP	6.91	POP	20.02	PLP	6.36	POL	3.39	OOL	1.76
PPS	1.21	POS	3.50	PLS	1.11	SLO	0.60	OLL	0.56
		PMO	0.22	PPL	1.17	SOL	0.30	LOL	0.14
		PPO	7.16	OSL	0.11	OOO	5.38		
		PSO	0.68	SPL	0.10	OPL	0.61		
		SOS	0.15	POO	20.54	MOL	-		
		SPO	0.63	SOO	1.81				
				OPO	1.86				
				OSO	0.18				
				PSL	-				
Others	0.16		0.34		0.19		0.15		0.22
Total	9.57		33.68		34.12		17.16		5.47

Note: M, myristic; P, palmitic, S, stearic; O, oleic; L, linoleic

1.1.3 Edible and Non-Edible Uses of Palm Oil

Palm oil is used in both edible and non-edible applications and 90 percent of palm oil and its products are used for edible purposes. Palm oil is used extensively in food preparation and manufacturing mainly as cooking and frying oils, or shortenings and margarine. Figure 1.3 provides examples on a number of palm-based food applications. The remaining 10% of palm oil and its products are used for non-edible applications, mainly in the soap and in oleochemical industries (Sarmidi *et al.*, 2009).

Figure 1.3 Variety of palm oil-based food products (Hai, 2002)

1.1.4 Palm Oil in Biodiesel

Biodiesel is a clean-burning diesel fuel produced from vegetable oils, animal fats, or grease. Biodiesel as a fuel gives much lower toxic air emissions than fossil diesel. In addition, it gives cleaner burning and has less sulphur content, and thus reducing emissions. Due to its origin from attractive resources, it is expected to compete with petroleum products in the future (Hayyan *et al.*, 2010).

Biodiesel can be chemically defined as a methyl ester which is prepared from triglycerides in vegetable oils by transesterification with methanol (Meher *et al.*, 2006). Biodiesel has been extensively researched using many different types of vegetable oils. Among these sources, palm oil is the cheapest vegetable oil due to its higher yield of approximately 5000 kg per hectare, compared with other vegetable oils, the maximum of which, for coconut oil is around 2250 kg per hectare. Therefore, it would be economically instinctive to believe palm oil as the feedstock for biodiesel production (Al-Zuhair *et al.*, 2007).

In Malaysia, palm oil is used in the production of biodiesel (palm oil methylester or palm oil diesel) for buses and cars due to the absence of sulphur and nitrogen. Furthermore, the use of palm oil is also advocated because it is assumed to dramatically reduce CO_2 emissions (Reijnders & Huijbregts, 2008).

1.1.5 Uses of Palm Oil Biomass

Huge quantities of biomass by-products, such as empty fruit bunches fibers, shells, fronds, and trunks are produced. These biomasses can be converted into many value added products. Palm oil biomasses can be transformed into three types of biomass energies, which are bio products, biofuels, and bio power (Kalinci *et al.*, 2011). Figure 1.4 shows the scope of biomass initiatives. Biofuels are used in the transportation sector. There are three types of biofuels that are bioethanol, bio diesel, and bio methanol. Palm oil based biofuels are ‘‘environmentally friendly’’ compared with fossil fuels which could cause the damage to the environment through emission of large quantities of green-house gases and pollutants (Chew & Bhatia, 2008). Biopower is the use of biomass to generate electricity. There are six major types of biopower systems: Direct firing, cofiring, gasification, pyrolysis, anaerobic digestion, and small modular systems. Most of the bio power plants use direct fired systems. In addition, gas and liquid fuels can be produced from biomass through pyrolysis. In pyrolysis biomass is heated in the absence of oxygen. The biomass then turns into a liquid called pyrolysis oil, which burns like petroleum to generate electricity (Bazmia *et al.*, 2010).

1.1.6 Electrochemical Studies Involving Palm Oil

The usage of palm oil in the electrochemical field has been investigated by different authors. Different electrochemical techniques have been used to treat and electroanalyze palm oil such as voltammetry, potentiometry, and chronopotentiometry, for different objectives. One of these objectives is to generate electricity using fuel cells.

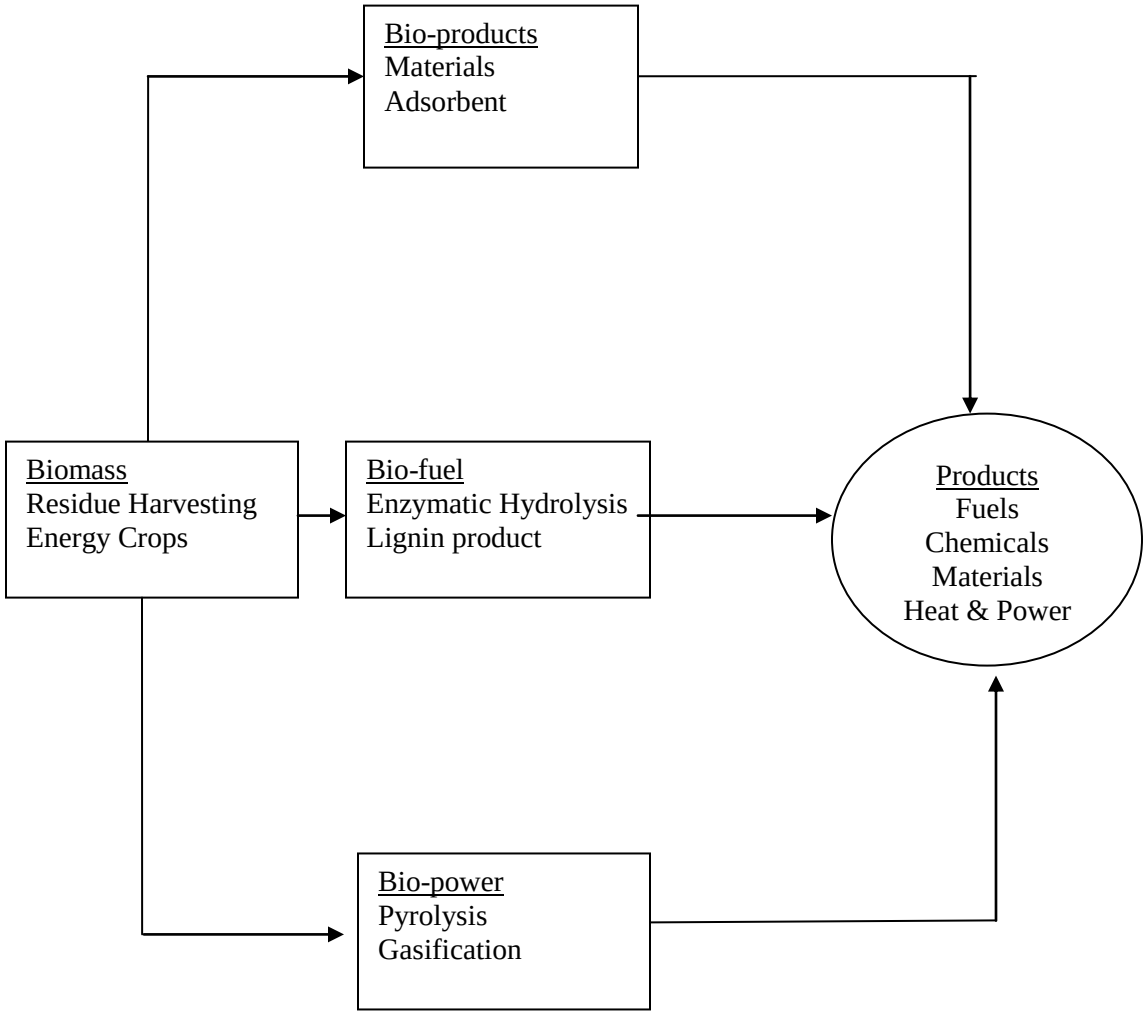


Figure 1.4 Biomass initiatives as renewable energy (Sumathi, *et al.*, 2008)

An electrochemical determination of vitamin E in palm oil has been reported (Atuma, 1975). The method involves saponification, extraction of the unsaponifiable material and direct voltammetric determination. Two indicator electrodes (glassy carbon and carbon paste) have been employed in this work for comparison purposes. The most important advantage of this method is its precision and the rapidity of analysis. The determination of the copper content of crude and hydrogenated palm oils has been investigated using a copper(II) ion-selective electrode with the direct potentiometric method. The method does not suffer from matrix effects and the reproducibility is reasonable. The apparatus assembly is simple, the running cost is low, and the capital cost is less for this method (Fung & Fung, 1978).

The determination of copper and lead in palm oil has also been achieved by stripping chronopotentiometry technique. The metal ions were concentrated as their amalgams on the glassy carbon surface of a working electrode that was coated with a thin mercury film. An ultrasonic bath was used for the extraction of copper and lead from the oil samples. The concentration of trace metals is an important criterion for the assessment of oil qualities with regard to freshness, maintenance properties, storage, and their influence on human nutrition and health (Cypriano *et al.*, 2008).

The electroanalytical possibilities of using Nafion–coated probes in cooking palm oil without any form of sample modification or dilution by conducting solvents has been investigated (Surareungchai & Kasiwat, 2000).

The electrochemical probe consists of co planar platinum working and counter electrodes, and a silver quasi reference electrode, was coated with a film of Nafion. It was demonstrated that the probe could be used to perform direct analysis of the antioxidant *tert*-butyl hydroquinone in cooking palm oils using differential pulse voltammetry.

An electro-Fenton pretreatment and biological oxidation has been used for the removal of recalcitrant contaminants present in palm oil effluent obtained from a food processing industry. Low molecular weight fatty acids were obtained at the end of electro-Fenton pretreatment and it was further degraded to CO_2 by biological oxidation. Electro-Fenton enables the successful increment of biodegradability index of the wastewater and plays an important role in wastewater management (Babu *et al.*, 2010).

Furthermore, amperometric enzyme electrodes have been constructed by adsorbing anionic royal palm tree peroxidase on spectroscopic graphite electrodes. The resulting H_2O_2-sensitive biosensors were characterized both in a flow injection system and in batch mode to evaluate its main bioelectrochemical parameters. The results indicate a uniquely superior characteristic of the biosensors, which due to the high stability of this enzyme in presence of H_2O_2 with an extremely high thermal and pH-stability (Alpeeva *et al.*, 2005).

An optical thin-film biosensor chip-based analytical technique has been proven to be a rapid, simple, specific, and sensitive method suitable for the detection of trace amounts of species-specific DNA from palm oil and has been demonstrated to be effective with other different vegetable oils (Bai *et al.*, 2011).

The multiwalled carbon nanotubes were synthesised by utilising palm oil as organic carbon sources at 700°C and then inserted electrochemically with Lithium. The charge/discharge test of Lithium/ multiwalled carbon nanotubes cells was performed under a galvanostatic mode. The irreversible capacity of the multiwalled carbon nanotubes was found to be relatively large due to formation of the passivation layer on the tube surface (Kudin *et al.*, 2009).

The supercritical water gasification of wet biomass from empty fruit bunch palm as a hydrogen production has been used to generate electricity using fuel cell. Supercritical water behaves like a non - polar organic single-phase solvent. It has been applied in the production of hydrogen rich fuel gas from wet biomass. The empty fruit bunch which has high moisture is just waste produced from a palm oil factory. The hydrogen produced by this process has been utilized to generate electricity using fuel cell which is an electrochemical device that produces electricity from a combined chemical reaction and electrical charge transport. Therefore, it offers potential benefits, efficiency, no emissions, and greenhouse gas reduction (Utomo *et al.*, 2006).

Palm oil biofuel cell has never been tried before but other edible oils (soy bean oil) biofuel cell have been reported (Kerr & Minteer, 2008).

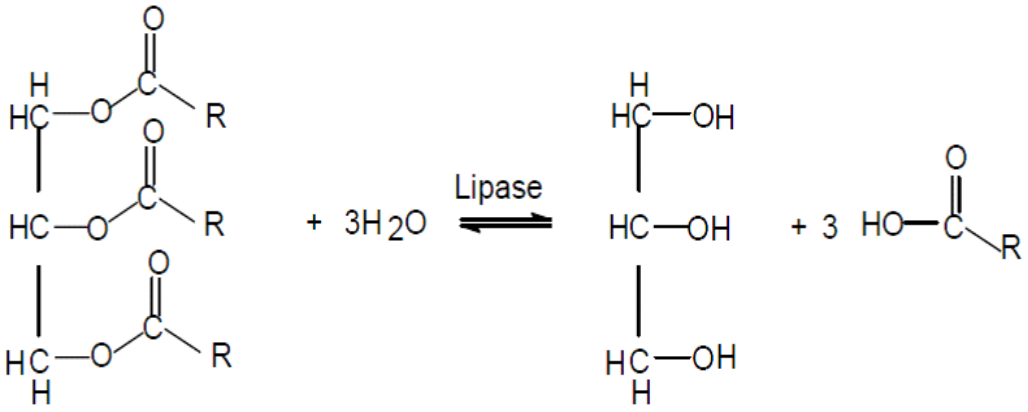
1.1.7 Hydrolysis of Palm Oil

Triglyceride the main component of natural oil or fat is converted stepwise into diacylglycerol, monoacylglycerol, and glycerol by hydrolysis accompanied with the liberation of fatty acid at each step (Beisson *et al.*, 2000). Hydrolysis of oil and fat is an important industrial operation; the products glycerol and fatty acids are widely used as raw materials in food, cosmetic, and pharmaceutical industries (Snape & Nakajima, 1996).

The Colgate-Emery process has been used for the hydrolysis of oil, in which pressurized steam at high temperature has been employed to hydrolyze ester bonds (Bamebey & Brown, 1948). This process not only consumed energy, but also affects the properties of fatty acids in the triacylglycerol mixtures, produce undesirable compounds such as ketones and hydrocarbons, and also undesirable colour impurities which have to be separated from the products (Al-Zuhair *et al.*, 2003).

Recently, enzymatic hydrolysis of triglycerides has gained increasing attention, which can be carried out at room temperature and atmospheric pressure making it energy efficient in comparison with the steam splitting process (Murty *et al.*, 2002). Furthermore, enzymes are biodegradable and consequently are less polluting than chemical catalysts (Cavalcanti-Oliveira *et al.*, 2011). Lipases are a class of hydrolyses enzymes that are primarily responsible for the hydrolysis of acylglycerides (Sharma *et al.*, 2001).

Lipases (EC 3.1.1.3) are serine hydrolases that do not require any cofactors (Singh *et al.*, 2008). It catalysed reactions that take place at the interface between the aqueous phase containing the enzyme and the oil phase (Lee *et al.*, 2006). Lipase is a polypeptide chain folded into two domains, the C terminal domain and the N-terminal domain which contain the active site with a hydrophobic tunnel from the catalytic serine to the surface that can accommodate a long fatty acid chain. The catalytic mechanism of lipases is centred on the active site serine. The nucleophilic oxygen of the active site serine forms a tetrahedral hemiacetal intermediate with the triacylglyceride. The ester bond of the hemiacetal is hydrolysed and the diacylglyceride is released. The active site serine acyl ester subsequently reacts with a water molecule. The acyl enzyme is then cleaved and the fatty acid is dissociated (Öztürk, 2001). A typical reaction catalyzed by lipases is shown in Figure 1.5.



Triacyl glycerol Glycerol Fatty acids

Figure 1.5 Hydrolysis of triacylglycerol by lipase

The exclusive features of lipases such as substrate specificity, regio-specificity and chiral selectivity drew great attention in both physiological and biotechnological aspects. Their main applications are in organic chemical processing, detergent formulations, synthesis of biosurfactants, the oleochemical industry, the dairy industry, the agrochemical industry, paper manufacturing, nutrition, cosmetics, and pharmaceutical processing (Salihu *et al.*, 2011).

Lipases can be derived from animal, bacterial, and fungal sources so they all tend to have similar three-dimensional structures (Saxena *et al.*, 2003). Many pure lipases, often obtained by recombinant technology and be purchased from enzyme suppliers. Table 1.5 summarizes commercially available lipases.

To date the lipase from yeast *Candida rugosa* is the most industrially used enzymes due to its high activity both in hydrolysis as well as synthesis, versatile catalytic reactions and broad specificities (Ratledge & Tan, 1990; Redondo *et al.*, 1995). The three-dimensional structure of *Candida rugosa* lipase (Öztürk, 2001) can be seen in Figure 1.6.

Table 1.5 Important Commercially Available Lipases (Doucet, 2007)

Origin	Code	Applications
mammalian origin		
human pancreatic lipase	HPL	
human gastric lipase	HGL	
porcine pancreatic lipase	PPL	organic synthesis, digestive aid
guinea pig pancreatic lipase	GPL-RP2	
fungal origin		
Candida rugosa	CRL	organic synthesis
Candida antarctica B	CAL-B	organic synthesis
Rhizomucor miehei	RML	cheese manufacturing
Aspergillus oryzae	AOL	cheese manufacturing
Penicillium camembertii	PEL	oleochemistry
Rhizopus delemar	RDL	oleochemistry
Rhizopus oryzae (phospholipase A1 activity)	ROL	oleochemistry
Rhizopus arrhizus	RAL	oleochemistry
bacterial origin		
Pseudomonas glumae	PGL	detergent enzyme, organic
Burkholderia cepacia	PCL/BCL	synthesis
Pseudomonas mendocina	PML	organic synthesis
Chromobacterium viscosum	CVL	detergents
Bacillus thermocatenulatus	BTL-2	organic synthesis

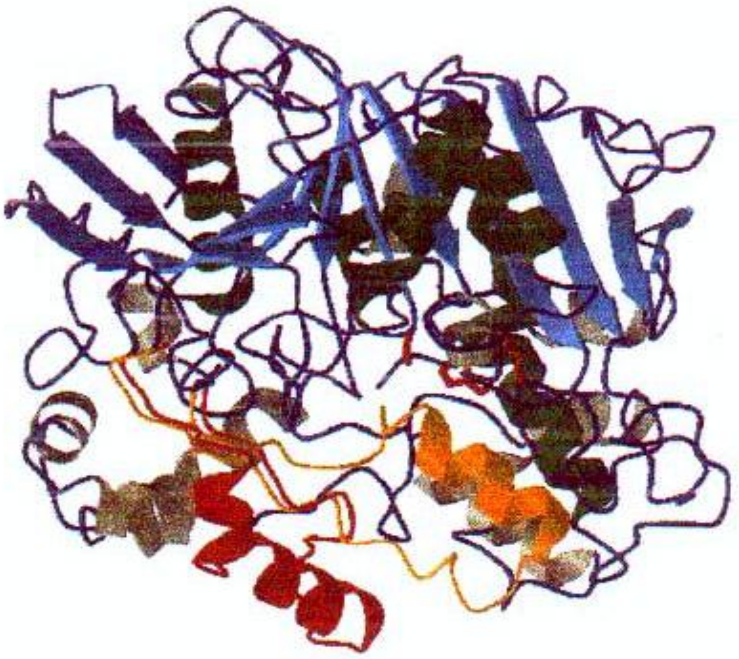


Figure 1.6 Three dimensional structure of *Candida rugosa* lipase (Öztürk, 2001)

The hydrolysis of palm oil, palm olein, and palm stearin by the commercial lipase from *Candida rugosa* has been reported (Khor *et al.*, 1986); palm oil and palm olein were found to be hydrolyzed at the same rate under the same conditions, whereas palm stearin was hydrolyzed much more slowly. The kinetics of *Candida rugosa* lipase hydrolysis of palm oil in lecithin/isooctane reverse micellar system has been also studied (Knezevic *et al.*, 1998), the reaction was found to obey Michaelis-Menten kinetics for the initial conditions. Five commercial lipases were compared for their ability to

hydrolyze palm olein in organic solvent in a two-phase system (H-Kittikun *et al.*, 2000). The results indicated that lipase from (*Candida rugosa*) showed the highest specific activity and achieved nearly complete hydrolysis.

The effects of enzymatic hydrolysis on crude palm olein by lipase from *Candida rugosa* have been also investigated (You & Baharin, 2006). Crude palm olein was hydrolyzed first to produce an oil rich in free fatty acids, then a comparison has been also made between crude palm olein and hydrolyzed crude palm olein for properties such as melting point, percentage of free fatty acids produced and viscosity. Hydrolyzed crude palm olein is found to be preferred in this hydrolysis process.

In this study we described the optimum conditions for the hydrolysis of refined palm oil using commercial *Candida rugosa* lipase. The effect of oil loading, enzyme loading, pH, temperature, and incubation time was investigated. The liberated fatty acids and glycerol from the hydrolysis of refined palm oil was used then as a substrate for different enzymes immobilized in ammonium modified Nafion membrane on the carbon electrode for potential biofuel cell applications.

1.2 Biofuel Cells

The energy-demanding bioelectronic devices require small power sources that are able to maintain operation over long periods of time at natural conditions. Miniaturized biofuel cells in the future can be used as alternative energy supply sources for nano-microelectronic devices and biosensors (Ramanavicius *et al.*, 2005). The concept of biofuel cells has been recognized for almost one century since the first

microbial biofuel cell was demonstrated in 1912. In the 1960s, NASA showed great interest in power generation from human wastes on the space shuttles (Kim *et al.*, 2006).

The production of electrical power from low-cost biofuels is an essential challenge in the energetics, since biofuels are renewable, sustainable, and reduce the demand for common fuel sources (Pizzariello *et al.*, 2002). The development of ever more miniaturized and complicated implantable biomedical devices represent the driving force for the design of small, reliable, biocompatible, and low power source systems biofuel cells are being developed for such applications (Barrière *et al.*, 2006). Biofuel cells are able of utilizing naturally existing biomass as fuel. They are an important substitute to conventional fuel cells and batteries that are besieged by non renewability, non implantability, size/weight, operating conditions (high temperature, acidity, and toxicity), waste issues, and logistics (Bilge *et al.*, 2009).

Biofuel cells belong to a special class of fuel cells where biocatalysts such as microorganisms or enzymes are employed instead of metallic inorganic catalysts (Sokic-Lazic & Minteer, 2008). Biofuel cells function by coupling two reactions, the oxidation of a biofuel (by microbes or enzymes) at the anode and the reduction of molecular oxygen to water at the cathode. The electrons travel from the anode to the cathode of the biofuel cell, where they are finally accepted by molecular oxygen. The availability of electrons and protons at the cathode foster the reduction of molecular oxygen to water. For an efficient system, the only byproducts of a biofuel cell should be carbon dioxide and water. This is an attractive feature of biofuel cell since the byproducts are non toxic. Biofuel cells would, therefore, be more environmentally friendly (Justin, 2001).

Biofuel cells reported in the literature are within two distinct categories: Biofuel cells that utilize the chemical pathways of living cells (microbial fuel cells) and those that employ isolated enzymes. Microbial fuel cells can achieve high efficiency in terms of conversion of chemical energy into electrical energy; however, problems associated with this approach include low volumetric catalytic activity of the whole organism and low power densities due to slow mass transport of the fuel across the cell wall (Moore *et al.*, 2004a).

During the past decades, oxidoreductases enzymes have concerned the efforts of many research groups in the environmental and biotechnological field due to their huge potential to eradicate pollutants and catalyze a great range of redox processes with no hazardous side effects (Fernández-Sánchez *et al.*, 2002). The application of redox enzymes for the targeted oxidation and reduction of particular fuel and oxidizer substrates at the electrode supports and the generation of the electrical current output is used for the development of biofuel cells (Ramanavicius *et al.*, 2008). In Figure 1.7 a schematic view of a biofuel cell is presented.

The first enzyme-based biofuel cell was reported in 1964 using glucose as the fuel (Yahiro *et al.*, 1964). Glucose is an ideal renewable fuel because it is produced by photosynthesis in plants such as sugar cane or corn which make it an attractive option as the fuel for portable fuel cells. Furthermore, glucose is plentiful in nature, cheap, environmentally benign, and easy to produce and handle (Ryu *et al.*, 2010).

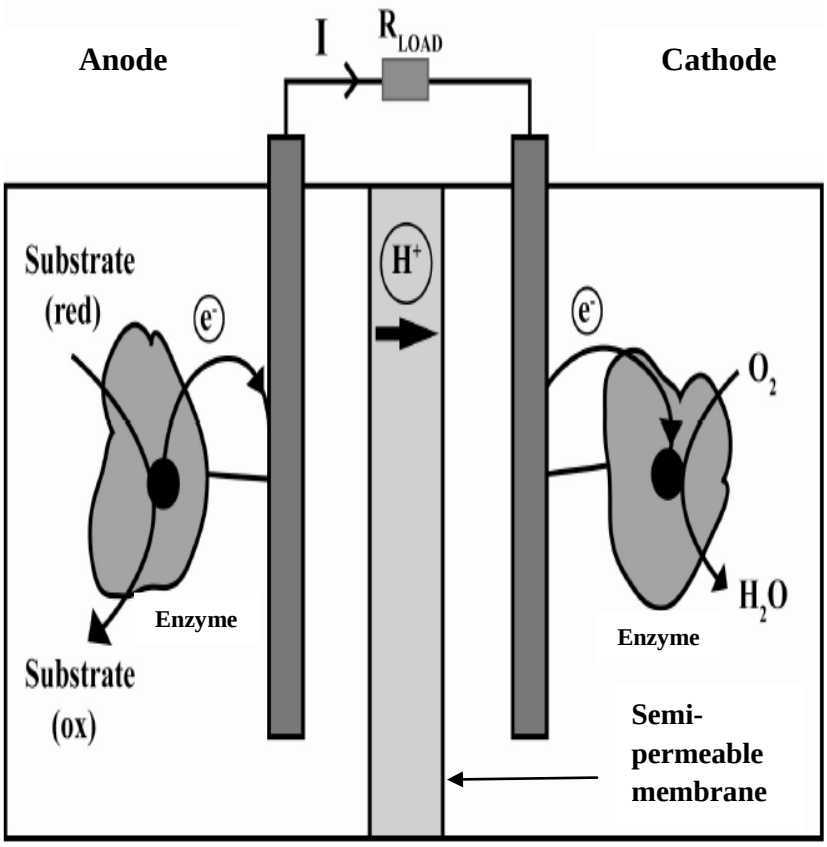


Figure1.7 A generalized scheme of an enzymatic biofuel cell, where a fuel is oxidized at the anode and molecular oxygen is reduced at the cathode (Coman, 2009)

While glucose is the most common fuel used by enzymatic biofuel cells, it is not essentially the best for all applications (Sokic Lazic *et al.*, 2010). There is a wealth of fuel choices such as pyruvate, its use was directed by its abundance and importance as a metabolic intermediate. Hydrogen as a non carbon containing fuel was known as a famous substrate for conventional fuel cells (Ivanov *et al.*, 2010).

Other fuels that have been used in enzymatic fuel cells are aliphatic alcohols such as methanol which is an attractive alternative to dihydrogen as the anodic fuel due to its ready availability and easy to transport and store (Palmore *et al.*, 1998). Ethanol has drawn further attention as a biofuel that can be produced by fermentation of biomass and is already commercially available for combustion engines. An enzymatic biofuel cell has been reported in which the anode and the cathode electrodes are both powered by ethanol and operate at ambient temperature (Ramanavicius, *et al.*, 2008). Glycerol is also an attractive fuel due to its high energy density, low vapour pressure, and low toxicity opposed to the latter alcohols. It is also plentiful due to the fact that it is a byproduct of biodiesel production (Arechederra *et al.*, 2007). Thus, the ability to oxidize such a higher order poly alcohols like glycerol would have a profound impact on the fuel cell market. The main fuels and the respective enzymes used for their bioelectrocatalytic oxidation are listed in Table 1.6.