EEFECT OF DIFFERENT ENZYMES AND OPTIMIZATION OF CULTURAL CONDITIONS ON XYLOSE PRODUCTION USING RESPONSE SURFACE METHODOLOGY (RSM)

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I declare that this thesis entitled "*Effect of Different Enzymes and Optimization of Cultural Conditions on Xylose Production Using Response Surface Methodology (RSM)*" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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This thesis is dedicated to all my love ones,
especially to Ibu & Ayah.
Thank you so much for the endless love & support.

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ABSTRACT

Xylose is found in the embryos of most edible plants. Xylan, the hemicellulose used as raw material in xylose production. Xylanase, the enzyme that is used to hydrolyzed the structure of xylan to produce xylose. This study aims to improve the production of xylose from Palm Oil Empty Fruit Bunch (POEFB) using enzymatic hydrolysis process. The effect of cultural conditions such as enzyme concentration and incubation time are used to study the production of xylose. Three different xylanases enzyme are used to produce xylose namely Multifect Xylanase, Multifect CX 12L and Optimase CX 72L. Multifect CX 12L was the best enzyme that yielded the most xylose.The optimization of the production of xylose is carried out by using response surface methodology (RSM) based on the central composite design (CCD). The optimal set cultural conditions for enzyme concentration are 50 U/g and 35.5 hours of incubation. Xylose production of 23.15 mg/ml was achieved by using these optimal conditions.

ABSTRAK

Xylose terdapat pada kebanyakan tumbuh-tumbuhan yang boleh dimakan. Xylan, merupakan hemiselulosa yang digunakan sebagai bahan mentah dalam penghasilan xylose. Xylanase, adalah enzim yang digunakan untuk menghidrolisasikan struktur xylan untuk menghasilkan xylose. Tujuan kajian ini dilakukan adalah untuk meningkatkan penghasilan xylose daripada hampas kelapa sawit (POEFB) yang telah diproses dengan menjalankan proses penghidrolisasian enzim. Pengaruh penghasilan xylose dikaji dengan mengendalikan keadaan pengkulturan seperti kepekatan enzim dan masa inkubasi. Penghasilan xylose dioptimumkan dengan menggunakan kaedah gerak balas permukaan (RSM) berdasarkan pada reka bentuk komposit pusat (CCD). Tiga jenis enzim xylanase digunakan dalam menghasilkan xylose iaitu *Multifect Xylanase*, *Multifect CX 12L* dan *Optimase CX 72L*. Enzim terbaik yang menghasilkan xylose yang paling tinggi adalah *Multifect CX 12L*. Keadaan pengkulturan yang optimum untuk penghasilan xylose adalah dengan menggunakan 50 U/g kepekatan enzim pada 35.5 jam masa inkubasi. Setelah menggunakan keadaan pengkulturan yang optimum ini, xylose yang terhasil adalah 23.15 mg/ml.

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LIST OF SYMBOLS/ABBREVIATIONS

ANOVA	-	Analysis of variance
CCD	-	Central composite design
DF	-	Dilution factor
DNS	-	Dinitrosalicylic Acid
EFB	-	Empty fruit bunch
g	-	Gram
HPLC	-	High performance liquid chromatography
hr	-	Hour
L	-	Litre
mg/ml	-	Miligram per millilitre
min	-	Minute
mM	-	Milimolar
mL	-	Mililitre
nm	-	Nanometer
OD	-	Optical density
OFAT	-	One factor at a time
POEFB	-	Palm oil empty fruit bunch
R	-	Regression
rpm	-	Round per minute
RSM	-	Response surface methodology
U/g	-	Unit per gram
w/v	-	Weight per volume
µL	-	Microlitre
µmol	-	Micromole
^{o}C	-	Degree celcius
%	-	Percentage

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CHAPTER 1

INTRODUCTION

1.1 Introduction

In Malaysia, Palm Oil fresh fruit bunch (FFB) is one of the most processed raw materials annually that can produce a wide range of applications. Ironically, any processed raw materials will end up producing waste. Due to the mass production of which originated from palm oil fresh fruit bunch, encourage a mass production of its waste, empty fruit bunch (EFB) which also called as biomass.

Recently, utilization of biomass resources has been the subject of various studies. Palm Oil Empty Fruit Bunch (POEFB) is one of the biomass materials, which is the by-product from the palm oil industry. Approximately 14 million ton of EFB biomass waste is generated annually throughout Malaysia. The fresh oil palm fruit bunch contains about 21% palm oil, 7% palm kernel, 15% fibre, 6% shell and 23% EFB (Ma *et al.*, 1993).

Many studies have been carried out on the utilization of POEFB such as in particle board, medium density fiberboard, pulp and composites. In general, the main structure of POEFB contains lignocellulosic. Regarding biomass utilization in lignocellulosic composites, it has been attributed to several advantages such as low density, greater deformability, less abrasiveness to equipment, biodegradability and low cost (Tay and Rozman, 2003).

There are three main components of lignocellulosic; lignin, cellulose and hemicellulose (Sreekala *et al*., 1997). Fortunately, various units of sugars in lignocellulosic can be derived from cellulose and hemicellulose, which are commonly xylose, arabinose, galactose, mannose and glucose (Fernando *et al*., 2005).

Huge amount of xylose in angiosperm lignocellulose results xylose the second most abundant sugar in nature. Xylose comprises only 15-35% of the total sugar in wood, but comprises 45% of the recoverable sugar (Jeffries and Alexander, 1987). Hence, it is practical and feasible to fully utilized xylose as it can be fermented to useful products including ethanol and xylitol (Cheng *et al*., 1981). Xylitol is recovered after xylose has been catalytically hydrogenated. This new sugar along with xylose itself, is a reducing sugar.

Large scale of bioethanol production is currently using crops such as sugarcane and sugar beet that contain sucrose which is directly fermentable. In Europe, wheat is a major substrate in such production of ethanol for portable spirit and fuel ethanol (Hanne *et al*., 2005). Besides, naturally occurring agricultural byproducts or wastes can be used in other industrial bioprocess for the production of value added products through SSF (Pandey *et al*., 2001).

Due to the major utilization of xylose in industries, it is important to determine what are the best resolution in producing the maximum production of xylose. For that reason, it is significant to screen which of those commercialized enzymes will produce the maximum product of xylose. Using enzymatic hydrolysis method, various types of hydrolases enzymes can be used such as cellulases, mannanases and xylanases (Gunda *et al*., 1970). Furthermore, the cultural conditions of these enzymes are crucial too in order to optimize the best production outcomes.

Based on report by Rahman *et* al., (2006), it is a potential source of xylose which can be used as a raw material for production of xylitol, a high value product. The increasing interest on use of lignocellulosic waste for bioconversion to fuels and chemicals is justifiable as these materials are low cost, renewable and widespread sources of sugars.

1.2 Problem Statement

In Malaysia, there is an abundant supply of palm press fiber and POEFB produced which are regarded as wastes and have not been utilized adequately. The abundance of POEFB was believed has to have created an important environmental issue such as fouling and attraction of pests. Loads of them are returned to plantation sites as composts. They are also used to be burnt at the mills but now the burning is practically banned. Partially, they are utilized for mulching and raw material of fertilizers. However, most of them are still not properly utilized.

Fortunately, Palm Oil Empty Fruit Bunch consist of 24% xylan and cellulose 43% (Rahman *et al*., 2006). Therefore, it can be consumed to produce xylose which has a major field of applications in industries and can be very useful to the environment and society at large.

1.3 Objective of Research

The main objective of this research is to produce the maximum production of xylose from palm oil empty fruit bunch (POEFB).

1.4 Scope of Research Work

In order to achieve the objective, scope of study was divided into three as the following:

i. To determine the best enzyme that can produce the most yield of xylose using enzymatic hydrolysis by feeding three types of commercial xylanases into the treated EFB, which are: (a) Multifect Xylanase, (b) Multifect CX 12L and (c) Optimase CX 72L.

ii. To study the effect of cultural conditions of the best enzyme chosen from (i) such as enzyme concentration and incubation time on xylose production.

iii. To optimize the cultural conditions of the enzyme chosen by using response surface methodology (RSM) on xylose production.

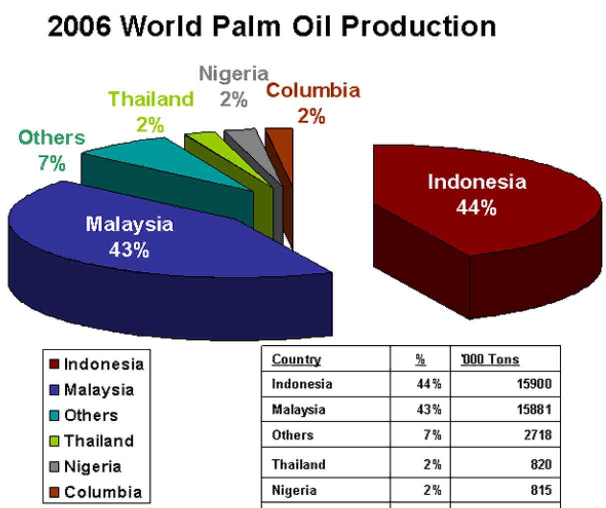
CHAPTER 2

LITERATURE REVIEW

2.1 Overview

Lignocellulose is a common biopolymer in the world. It is considered as one of the most important carbon sources on this planet (Qiu and Wei, 2007). Almost 60 % of plant biomass produced on earth is lignocellulose (Tengerdy and Szakacs, 2002). 75% of this lignocellulose is composed of carbohydrates and in the near future, it will become an essential source for fermentable carbohydrates (Henning *et al.*, 2007). Malaysia is one of the largest palm oil producers in the world (Figure 2.1), where more than 14 million tonnes of empty fruit bunch (EFB) which are considered as a lignocellulosic waste was produce annually and the number keeps increasing 5% yearly. Abundance of EFB was believed has to have created an important environmental issue such as fouling and attraction of pests (Law *et al.*, 2007). Therefore, this lignocellulose should be viewed as a local resource, which has the potential to be valuable feedstock in many processes, including the possibility of conversion to sugar that could be converted into ethanol via microbial fermentation. Besides, a new transportation fuels are badly needed to reduce our heavy dependence in imported oil and it can reduce the release of greenhouse gases that cause global climate change. Linocellulose biomass is the only inexpensive resource that can be used for sustainable production of the large volumes of liquid fuels.

Types of sugar that could be produced are xylose, arabinose, mannose and glucose which have their commercial value in pharmaceutical industries, food industries and other industries. Figure 2.1 and Table 2.1 shows the world production of palm oil in 2006 and 2008 respectively.



Country	%	'000 Tons
Indonesia	44%	15900
Malaysia	43%	15881
Others	7%	2718
Thailand	2%	820
Nigeria	2%	815
Columbia	2%	711
Source Data: Oil World, GAPKI		

Figure 2.1: 2006 World Palm Oil Productions.

Table 2.1: 2008 World Palm Oil Productions

Palm oil production, 2008	
1 Indonesia	19,700,000
2 Malaysia	17,400,000
3 Thailand	1,400,000
4 Colombia	830,000
5 Nigeria	820,000
6 Papua New Guinea	425,000
7 Ecuador	340,000
8 Cote d'Ivoire	320,000
9 Costa Rica	285,000
10 Congo, Democratic Rep	175,000
11 Cameroon	165,000
12 Honduras	165,000
13 Guatemala	155,000
14 Ghana	120,000
15 Brazil	110,000
16 Philippines	70,000
17 Angola	58,000
18 Venezuela	54,000
19 Guinea	50,000
20 India	50,000
Figures in metric tons. Data derived by mongabay.com from the USDA Foreign Agricultural Service.	

Suitable forest area for oil palm, 2008	
1 Brazil	2,283,000
2 Congo, Democratic Rep	778,000
3 Indonesia	617,000
4 Peru	458,000
5 Colombia	417,000
6 Venezuela	150,000
7 Malaysia	146,000
8 Papua New Guinea	144,000
9 Suriname	101,000
10 Bolivia	90,000
11 Cameroon	83,000
12 Gabon	81,000
13 Guyana	81,000
14 French Guiana	70,000
15 Congo, Republic of	66,000
16 Ecuador	55,000
17 Philippines	31,000
18 Myanmar	25,000
19 Thailand	24,000
20 Laos	13,000
Figures in sq km. Data derived by mongabay.com from the Woods Hole report: Readiness for REDD	

Based on Figure 2.2, xylose production can be employed by pretreatment of lignocellulose followed by saccharification. Xylose can also be further processed to produce furfural and xylitol which has broad application in industries. Figure 2.2 shows the general process in lignocellulose bioconversion into value-added bioproducts (Howard *et al.*, 2003).

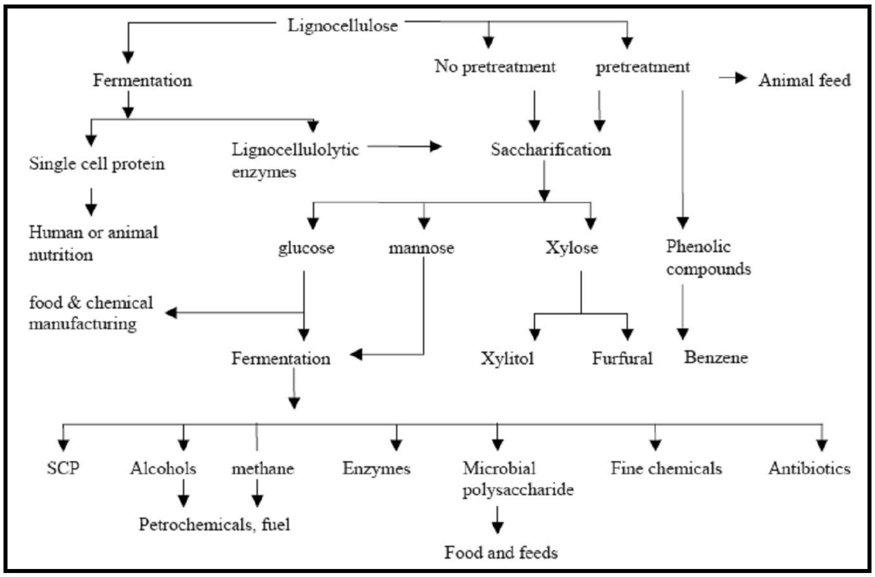


Figure 2.2: General process in lignocellulose bioconversion into value-added bioproducts (Howard *et al.*, 2003).

2.2 Palm Oil Empty Fruit Bunch (POEFB)

Oil palm is originated in the tropical forest of West Africa and it is one of the most economical and very high-potential lasting oil crops. It belongs to the species of *Elaeis guineensis* under the family *Palmacea*. EFB is important type of fibrous materials left in the palm oil mill. It was obtained after the removal of oil seeds from fruit bunch for oil extraction. Average yield of EFB fiber is about 400 g per bunch (Sreekala *et al.*, 1997). Figure 2.3 shows the photo of a palm oil EFB.

Figure 2.3: Photograph of an oil palm empty fruit bunch.

Traditionally, EFB have always been burnt and their ashes were recycled into the plantation as fertilizer. However, due to the pollution problem, burning of EFB has been discouraged. The EFB is now used mainly as mulch and fertilizers.

Additionally, if EFB is placed around young palms, it helps to control weeds, prevents erosion and maintains soil moisture. However, due to the current labor shortage, the transportation and distribution of EFB in the field is getting more expensive.

Recently, EFB has been investigated as a raw material for building materials, solid fuel pellets, chemical products, particleboard, fibreboard and pulp and paper (Ridzuan *et al.*, 2002).

Generally, the fresh oil palm fruit bunch contains about 21 % palm oil, 6-7 % palm kernel, 14-15 % fibre, 6-7 % shell and 23 % EFB (Umikalsom *et al.*, 1997). Annually, as mentioned before, Malaysia produces almost 14 million tonnes EFB, 0.4 million tonnes fiber and 0.5 million tonnes shell as shown in Figure 2.4 below.

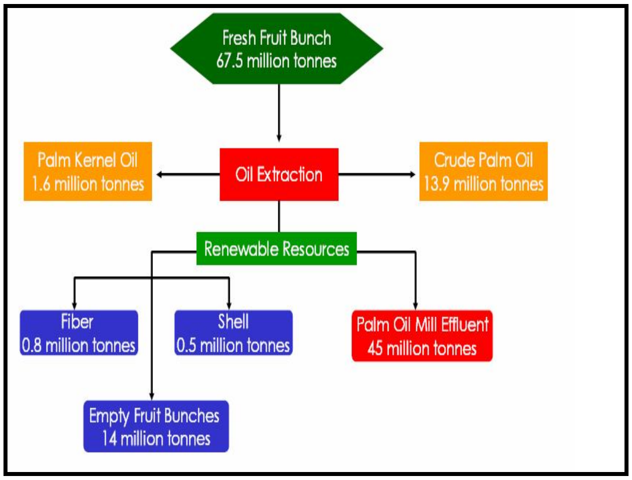


Figure 2.4: Types of biomass and quantity produced

2.3 Structure of Lignocellulosic Biomass

Main components of most lignocellulose materials are lignin, cellulose and hemicellulose. Each of this part plays a role in protecting each other and strengthens the plant structure. Figure 2.5 shows the structure of lignocellulose.

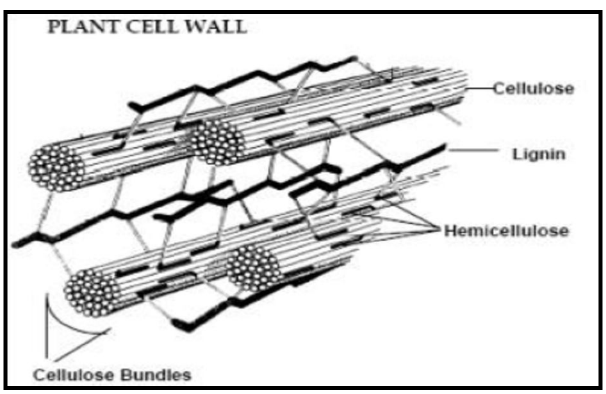


Figure 2.5: Structure of lignocellulose materials.

Distribution of lignin, cellulose and hemicelluloses as well as the content of the different sugars of the hemicelluloses varies significantly between different plants (Henning *et al.*, 2007). Figure 2.6 shows the typical plant cell wall's distribution of lignin, cellulose and hemicelluloses.

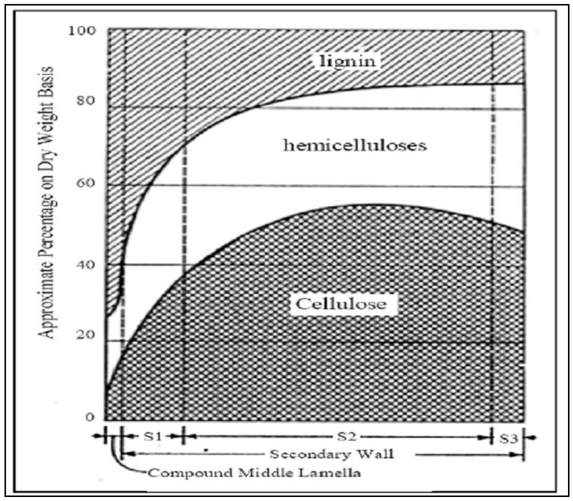


Figure 2.6: Distribution of lignin, cellulose and hemicellulose in a typical plant cell wall.

2.3.1 Lignin

Lignin is most abundant in the middle lamella and decreases with increasing distance into the fiber cell wall. It can be describe as a complex polymer of phenylpropane units, which are cross linked to each other with a variety of different chemical bonds giving the cell wall its main mechanical strength. It bound to xylans by an ester linkage to 4-O-methyl-D-glucoronic acid residues. Lignin is formed in vascular plant cell walls by the oxidative coupling of several related phenylpropanoid precursors such as coniferyl alcohol, sinapyl alcohol and p-hydroxycinnamyl alcohol (Haixuan *et al.*, 2002). Figure 2.7 shows the common structure of lignin.