THE TREATMENT OF OIL PALM EMPTY FRUIT BUNCH FIBER FOR SUBSEQUENT USE AS SUBSTRATE FOR CELLULASE PRODUCTION BY *ASPERGILLUS TERREUS* SUK-1

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A thesis submitted in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering

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I declare that this thesis entitled "*The treatment of oil palm empty fruit bunch fiber for subsequent use as substrate for cellulase production by Aspergillus terreus SUK-1*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ....
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Date : 20 November 2006

Special dedication to my mum and dad that always inspire, love and stand besides me,
my supervisor, beloved friends, fellow colleagues
and all faculty members.

For all your love, care, support and believe in me.

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ABSTRACT

Cellulases are important in industrial enzyme which is a main part in industrial process such as in bio-bleaching and bioethanol production. Nowadays, our country encourages the production of bioethanol from the biomass material such as oil palm empty fruit bunch fiber in order to enhance the resources of ethanol production and reduce the cost.In this project, oil palm empty fruit bunch fiber was used as a substrate for the production of cellulase enzyme by *Aspergillus terreus* SUK-1. In order to enhance the cellulase production, pretreatment on substrate are performed with different type of methods. Comparison results of cellulase enzyme production are determined between the substrate which is pretreated with acid and alkaline pretreatment. The effect of steam explosion on the substrate also was identified in shaking flask fermentation. From the experimental work, it was found that the highest xylanase and carboxymethylcellulase activity was in fermentation of acid treated substrate than alkali treated substrate and untreated substrate It also found that by autoclaved the substrate with chemicals produced the highest cellulase enzyme with value 0.167836U/ml for xylanase and 0.3534U/ml for carboxymethylcellulase.

ABSTRAK

Sellulase sangat penting dalam industri penghasilan enzim dan memainkan peranan penting dalam industri pembuatan seperti pembuatan kertas, peluntur biologi dan penghasilan bioethanol. Negara kini sedang meniggalakkan penggunaan bahan buangan seperti hampas kelapa sawit dalam meningkatkan penghasilan enzim sellulase dalam masa yang sama mengurangkan kos. Dalam projek ini, hampas kelapa sawit digunakan sebagai sumber karbon dalam penghasilan enzim dari *Aspergillus terreus* SUK-1. Untuk meningkatkan penghasilan enzim, substrate telah dirawat melalui beberapa kaedah. Keputusan telah dikaji antara penggunaan sumber karbon yang dirawat dengan bahan kimia dan tidak menggunakan bahan kimia. Selain itu, kesan letupan stim juga dikaji melalui aktivity enzim. Keputusan dari experiment ini telah menunjukkan bahawa penghasilan sellulase enzim pada tahap tinggi adalah dengan penggunaan substrate yang telah melalui process letupan stim dengan bacaan 0.167836 U/ml untuk xylanase dan 0.3534 U/ml untuk karboxymethylcellulase.

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LIST OF SYMBOLS

Å	-	Angstrom
β	-	Beta
μ	-	Micro
α	-	Alfa
°	-	Degree

LIST OF APPENDICES

APPENDIX	TITTLE
A	Standard curve of glucose
B	Standard curve for xylose
C	Cellulase enzyme at difference chemicals treatment
D	Cellulase enzyme at different chemical treatment with autoclaved
E	Comparison of Cellulase activity in different chemical and physical pretreatment

CHAPTER 1

INTRODUCTION

1.0 Introduction

Cellulase is important in in textile manufacturing, pulp and paper and food industry. Cellulase is a complex mixture of cellobiohydrolases, endoglucanases and beta glucosidases. It is important to convert complex carbohydrate present in lignocellulosic biomass into glucose. In fermentation, glucose will be converted into ethanol, butanol, acetone and other chemical composition which is using as biofuels (Gadgil *et al.,* 1995)

In Malaysia, oil palm as a main part to support the food industry to produce edible oil. Beside that, oil palm empty fruit bunch is a by product left in the palm oil mill. This residue may causes environmental pollution problems and spread diseases. Other researches stated that oil palm empty fruit bunch is a lignocellulosic source which is available as a substrate in cellulase production. The application of cellulase to produce bioethanol was handicap by the high cost. The use of empty fruit bunch, wastage from oil palm will reduce the cost of enzyme production comparable other carbon sources (Akamatsu *et al*., 1987). Nowadays, numerous researches have been carried out with microorganisms such as bacteria and fungi. It is included in cellulase production. In many cases has been proved that cellulase are produced by a wide range of microorganisms such as *Trichoderma* and *Penicillium* (Rajoka and Malik, 1990).

1.1 Problem Statement

In Malaysia, oil palm is a major agricultural industry which is a main source of edible oil. However, palm oil mill produce a large amount of solid waste called biomass. Biomass from plant are the main resources which renewable and sustainable resources available in production of ethanol and other chemicals. Biomass contributes approximately 14% of the total global final energy demand. It is included for cooking, heating and electricity. On the other hand, bioethanol is a new industry which is an alternative source of fuel. But the application of cellulase to produce bioethanol was handicap by the high cost. The use of waste such waste and low cost cellulosic biomass such as oil palm empty fruit bunch would significantly reduce the cost of enzyme production. In addition, the research outcome with new methods will increase the cellulase production. For example the pretreatment on substrate with acid and alkali will encourage and improve the production of enzyme.

Commonly, the biomass material uses as a substrate in cellulase production are not practical in Malaysia such as wheat straw and corn stoves. Thus, the enzyme industry is not performed well in Malaysia. Oil palm empty fruit bunch is a new renewable biomass that not widely use as a substrate. By doing the research on oil palm empty fruit bunch as a substrate with suitable methods and optimum condition, the new invention will arise and cellulase will be produced in the effective and efficient. Then, the economy in agriculture will be encouraged.

Nowadays, our country was face on the environmental problem that was caused by the fuels from transportations and the pollution of biomass material. The problems become worse when the sources of fuel are decreasing year by year. The production of bioethanol is an alternative way to solve the problem and become a new industry which is available in production of fuel. On the other hand, bioethanol can improve air quality by reducing carbon monoxide emission level.

1.2 Objective

The objective of this research is to determine the rate of production cellulase enzyme from *Aspergillus terreus* SUK-1 using oil palm empty fruit bunch fiber obtained from palm oil waste as a substrate which had been treatment in acid and alkali.

1.3 Scope of Research

3 scopes are determined in order to achieve the objective

1. To study the effect of xylanase and carboxymethylcellulase production by pretreated substrate with acid and alkali.
2. To study the effects of xylanase and carboxymethylcellulase production between the autoclaved substrate and non-autoclaved substrate
3. To determine an effective pretreatment in order to produce the maximum yield.

CHAPTER 2

LITERATURE REVIEW

2.1 Biomass in Malaysia

Biomass defined as full range of plant and plant derived materials. Biomass also describe as a renewable fuel resources which cover almost any biologically degradable fuel from farmyard manure through industrial liquid effluents and solid waste, agro industrial and forestry waste. Commonly, biomass from plant comprised of cellulose, hemicellulose, and lignin. Nowadays, biomass from plant are the main resources which renewable and sustainable resources available in production of ethanol and other chemicals. Biomass contributes approximately 14% of the total global final energy demand. In is included for cooking, heating and electricity.

In Malaysia, oil palm is a main resource in production of edible oil which is important in food industry and as a daily usage. According to Pusat Tenaga Malaysia, PTM 2005, the waste from oil palm consist fiber, empty fruit bunch, shell, POME, and trunks. The waste from palm oil are higher than waste from other plants such as rice, sugarcane and wood. In 2003, biomass from oil palm consists 14 million tonnes of oil palm empty fruit bunch; 8 million tones palm kernel shell and 5 million tones mesocarp fiber.

2.1.1 Bioenergy in the world context

Biomass has a significant role to play in solving the world's energy needs. Biomass combustion is carbon neutral. The carbon dioxide released in combustion is recycled by trees and crops which may provide fuel for the future. By utilising biomass as a fuel instead of a non renewable fossil fuel, the net carbon dioxide released into the atmosphere is deemed to be reduced. Biomass is capable of replacing fossil fuels in order to provide electrical power and generate heat in those areas where it is abundantly available. In order for biomass to be widely accepted, overall cost factors have to be fully analysed. Comparing the use of biomass to other established fossil fuels has to be taken into account, together with the investment and infrastructure already in place. Incorporating bioenergy into a holistic framework which assesses the total cost including the environment shows that biomass can be a competitive energy source (Shell, 1999).

2.2 Oil Palm Empty Fruit Bunch

The oil palm (*Elaeis guaineensis*) has become the most important economic plantation crop in Malaysia. There is the major source to produce edible oil which is extracted from fruits (Lua and Gua, 1998) However; palm oil mills produce a large amount of solid wastes. The remainder of the oil palm consists of huge amount of lignocellulosic materials such as oil palm fronds, trunks and empty fruit bunches. The residues contain 7.0 million tonnes of oil palm trunks, 26.2 million tonnes of oil palm fronds and 23% of Empty Fruit Bunch (EFB) per tonne of Fresh Fruit Bunch (FFB) processed in oil palm mill.

Figure 2.1: oil palm empty fruit bunch fiber

2.2.1 Composition of oil palm empty fruit bunch

Empty Fruit Bunch is composed of 45-50% cellulose and about equal amounts (25-35%) of hemicellulose and lignin (Deraman 1993). Due to oil palm empty fruit bunch is available in large quantities and has fairly high cellulose contents. So empty fruit bunch fiber is appears to be a potential substrate for enzyme and other chemical production. (Deraman, 1993)

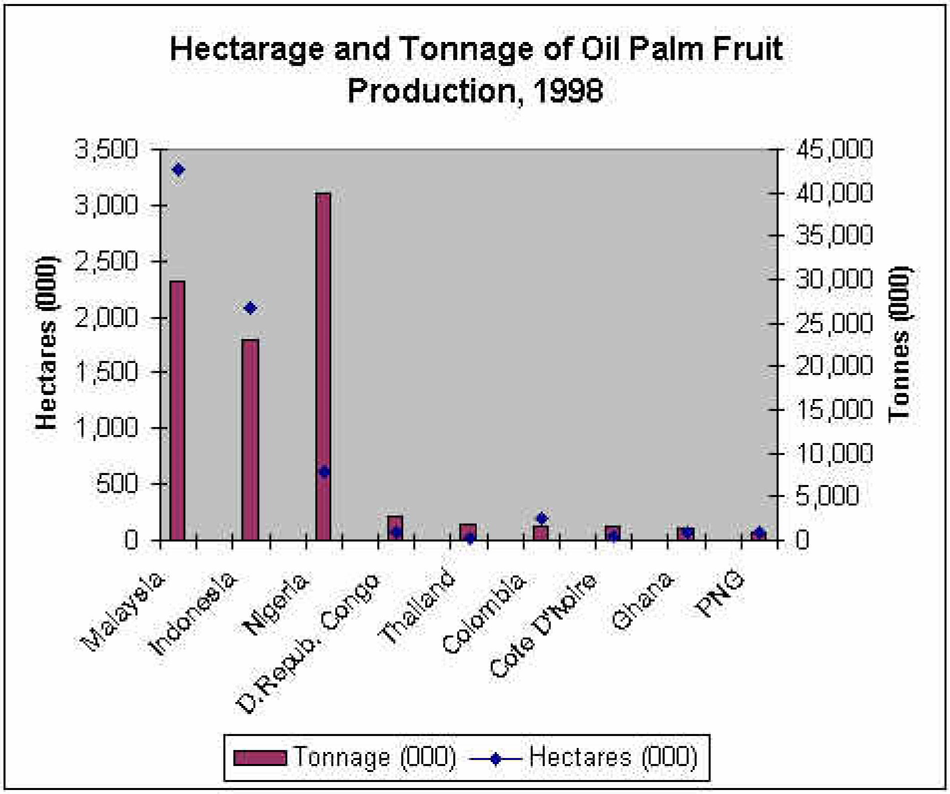

Figure 2.2 Graph of Hactarage and Tonnage of Oil Palm Fruit Production, 1998

The figure showed the production of oil palm fruit in 1198. The growth of the oil palm industry is rapid with over 8.8 million hectares under production in 1998. Oil palm fruit production has risen at a huge rate from 11 million tonnes in 1990 to 94 million tonnes in 1998, FAO 1999. By next year yields will be over 100 million tonnes, FAS online 1990, 43-45% of this will become mill residue in the form of Empty fruit bunches (EFB), shell and fibre. The chart below illustrates the worldwide differences in production and the hectarage involved.

2.3 Cellulose

Molecules of cellulose are organized into long, unbranched microfibrils that give support to the cell wall. Cellulose is the most abundant substance found in the plant kingdom. Plants are made up of thousands of individual cells, each of which has an external skeleton in the form of a cell wall. The cell wall is made up of a mesh of cellulose surrounded and interlinked by supporting materials.

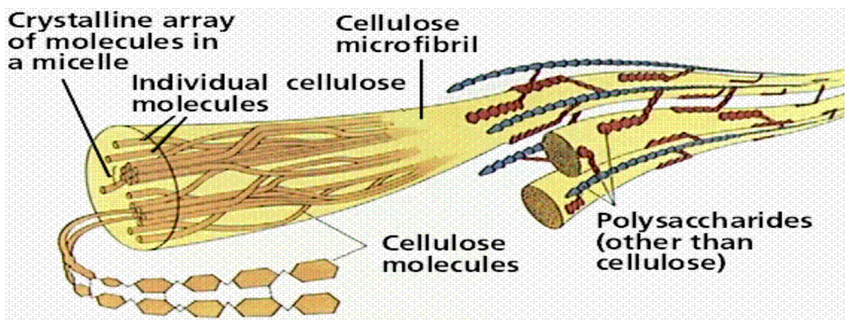

Figure 2.3: cellulose in plant structure (Bailey, 1986)

The figure 2.2 showed the cellulose molecules in plant structure. Cellulose is the major component of all plant material, comprising up to half of the plant's dry weight. Cellulose molecules are long, thin and straight, being made up of unbranched chains of D-glucose subunits with a molecular weight ranging from 50,000 to over 1 million (Bailey, 1986).

2.3.1 Structural unit of cellulose

Glycosidic linkage in cellulose occurs between the 1 and 2 carbons of successive glucose units, the subunits are bonded differently than in amylase. That is difference in structure is significant. While, many microorganisms, plants and animals process the necessary enzymes to break (hydrolyze) the a-1, 4-glycosidic bonds which are found in starch and glycogen, few living creature can hydrolyze the B-14-bonds of cellulose. One of the common products of enymatic cellulose hydrolysis is cellobiose, a dimmer of two glucose units joined by the B-1, 4-glycosisic linkage

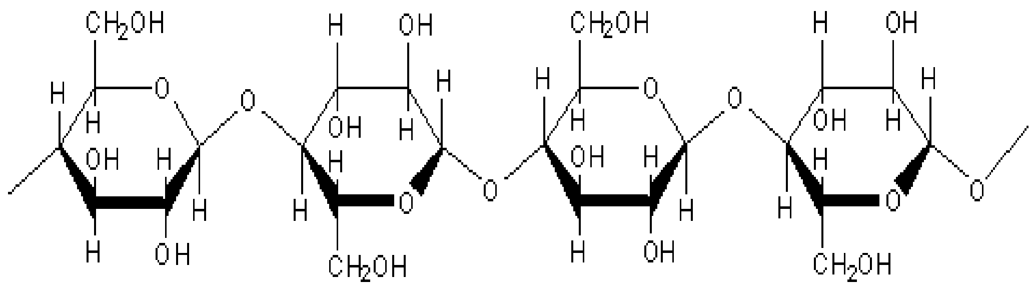

Figure 2.4: molecule of cellulose (Bailey, 1986)

The figure showed the molecule of cellulose in plant cell. Cellulose is a polymer, or more specifically a polysaccharide, which is made of more than 3,000 glucose units. A polymer is simply a larger molecule consisting of many smaller, repeated subunits; in this case glucose is the subunitIn

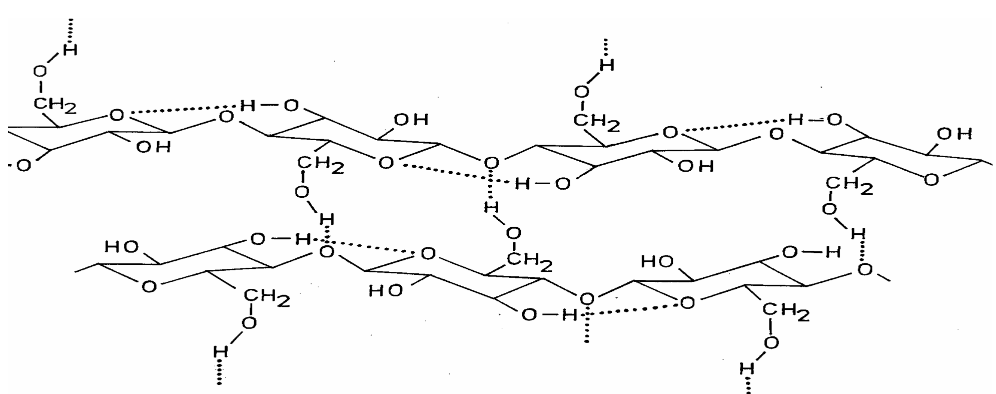

Figure 2.5: Schematic view the hydrogen bonding between glucose residues (Bailey, 1986)

Figure 2.5 shows the schematic view the hydrogen bonding between glucose residues. ß-1, 4-Connected glucose residues are trapped in a plane of the crystal by hydrogen bonds. Each glucose residue is turned by 180° towards its neighbours, making cellobiose the smallest subunit. This picture explains why cellulases active on crystalline cellulose usually release cellobiose (and not glucose).

2.3.2 Functionality

Cellulose many uses as an anticake agent, emulsifier, stabilizer, dispersing agent, thickener, and gelling agent but these are generally subsidiary to its most important use of holding on to water. Another important derivative of cellulose is carboxymethylcellulase (Chaplin, 2004).

2.4 Cellulase Enzyme

Cellulase is important in industrial process such as bioethanol, pulp and paper and biobleaching. Usually, cellulase enzyme can be produce by using symbiotic bacteria in the ruminating chamber which is reacting with substrate (cellulose*). Trichoderma reesei* extensively studied cellulose enzyme complex. This enzyme converts crystalline, amorphous, chemically derived cellulose quantitively to glucose. *Trichoderma longbrachiatum* comprised of an enzyme complex consisting of cellulose, a glucosidase, cellobiohydrolase and glucanase. This complex converts to beta dextrine and D glucose.

2.4.1 Cellulase enzyme in industry

Cellulase use in pulp and paper industry. Lignocellulosic materials with lignin-degrading fungi as a main part use to manufacture the pulp. Enzymes (laccase) also are used to improve physical properties of fibers, enhance pulp fibrillation and ultimately improve paper strength. Amylases enzyme from cellulase complex important in the coating process of paper (Fan *et al*., 1987). With the high-alkali concentration during the kraft cook, part of the xylan is dissolved in the pulping liquor; while short-chain xylan precipitates in a more or less crystalline form on the surface of cellulose microfibrils. This xylan forms a barrier against effective extraction by chemicals of the residual brown-coloured lignin from the fibres. As a result, quantities of chlorine or chlorine-containing compounds are required to be used for effective reduction in the kappa number, and increase in pulp brightness. Enzymatic solubilization of the hemicellulose settled on the pulp fibres would be an 'environmentally compatible' technology to improve the accessibility of the brown lignin to chemical bleaching together with substantially reduced quantities of bleaching chemicals required to achieve the same degree of bleaching and brightness.

Cellulase is a common enzyme adds in detergent to improve the performance of detergent. Dirt comes in many forms and includes proteins, starches and lipids. In addition, clothes that have been starched must be freed of the starch. Using detergents in water at high temperatures and with vigorous mixing, it is possible to remove most types of dirt but the cost of heating the water is high and lengthy mixing or beating will shorten the life of clothing and other materials. The use of enzymes allows lower temperatures to be employed and shorter periods of agitation are needed, often after a preliminary period of soaking. Detergent enzyme can be reducing the cost and safe to use. On the other hand, it is more environmental friendly and suitable for daily usage.

2.4.2 Xylanase enzyme

Xylanase belong to glucanase enzyme family and characterize by their ability to break down various xylans to produce short chain xylooligosaccharides. Xylanase is supply as sodium / potassium phosphate and glycerol in liquid form (Shah, 2005)

(Endo-1, 4-Beta-xylanase) from Trichoderma sp has a pI of 9.0 and is produced by fermentation. Xylanase consists of 190 amino acids and has a molecular weight of 21 kD. Xylanases belong to the glucanase enzyme family and are characterized by their ability to break down various xylans to produce short-chain xylo-oligosaccharides.