THE EFFECT OF BASE PRE-TREATMENT ON LIGNIN DEGRADATION AND STRUCTURE OF EMPTY FRUIT BUNCHES (EFB) FOR BIOHYDROGEN PRODUCTION

MOHAMMAD NIZAM BIN MANSOR

CHEMICAL ENGINEERING
UNIVERSITI TEKNOLOGI PETRONAS
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By

MOHAMAD AIZAM BIN MANSOR
7776

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Universiti Teknologi PETRONAS
Bandar Seri Iskandar
31750 Tronoh
Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

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Approved by,

(ASSOC. PROF. DR. ZAKARIA MAN)

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK
July 2009

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

NAME: MOHAMAD AIZAM BIN MANSOR

ID: 7776

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ABSTRACT

Alkaline/base pre-treatment has its own effect(s) on the cellulose, hemicelluloses and lignin, the three main components of lignocellulosic biomass. Many chemical treatments have been used for lignin removal and destruction of the lignin crystalline. From these chemicals, acids, gases, oxidizing agents are able to increase digestibility, but are not popular as alkalis because it is very economic, simpler processes and less degradation favor alkalis as chemical pre-treatment agents. The objective of this paper is to investigate on the effect of alkaline/base pre-treatment using Sodium Hydroxide (NaOH) on the lignin degradation and the structure of Empty Fruit Bunches (EFB) based on the concentration of total reducing sugars in hydrolysis process to improve its digestibility for bio-hydrogen production. Batch experiments were conducted using NaOH and Acid Sulfuric (H_2SO_4) as the reaction catalyst at different NaOH concentration, different particle size at low temperature condition. The SEM micrographs of pretreated EFB fiber showed tremendous changes in the morphology structure of the fiber. 20% of NaOH was an economical base for the treatment purpose. The crystallinity of the reacted fibers was decreased as concentration of NaOH increased. Besides, this study is also to investigate the effects of alkalis pretreatment towards the characteristic of feedstock for gasification process to produce bio-hydrogen. In addition, this study also reviews chemical pretreatment using alkaline/base method from the perspectives of its potential use with EFB application to bio-hydrogen.

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LIST OF ABBREVIATIONS

NaOH	Sodium Hydroxide
H_2SO_4	Sulfuric Acid
SEM	Scanning Electron Microscopy
EFB	Empty Fruit Bunches

CHAPTER 1

INTRODUCTION

Dependence on fossil fuels as the main energy sources has led to serious energy crisis and environmental problems, i.e.fossil fuel depletion and pollutant emission. The increasing energy demands will speed up the exhaustion of the finite fossil fuel. Moreover, combustion of fossil fuel produces substantial greenhouse and toxic gases, such as CO2, SO2, and other pollutants, causing global warming and acid rain. In response to the two problems stated above, continuous effort has been made in exploration of clean, renewable alternatives for a sustainable development. Biomass is one of the most abundant renewable resources. It is formed by fixing carbon dioxide in the atmosphere during the process of plant photosynthesis and, therefore, it is carbon neutral in its lifecycle. Biomass has been used for centuries. Currently, biomass contributes about 12% of today's world energy supply, while in many developing countries it contributes 40–50% energy supply [M.A.H. Abdallah *et al.* 1999]. Biomass research is recently receiving increasing attention because of the probable waste-to-energy application. One of the major drawbacks is the low efficiency of utilizing biomass. Alternatively, converting biomass into gaseous and aqueous fuels, electricity and specially hydrogen is possibly a more efficient way of biomass utilization. This project aims to give an overview of initial stage methods of producing hydrogen from biomass which is chemical pretreatment and its development potential.

1.1 Background

Oil palm production is a major agricultural industry in Malaysia. It contributes about US$ 7.3 billion in export earnings each year, mostly from the export of palm oil. In total, about 90 million mt of renewable biomass (trunks, fronds, shells, palm press fiber and the empty fruit bunches) are produced each year. The empty fruit bunches (EFB) represent about 9% (Lim 2000) of this total. They are the residue left after the fruit bunches are pressed at oil mills, and the oil extracted (Ma *et al.* 1993; Kamarudin *et al.* 1997). EFB is a suitable raw material for recycling because it is produced in large quantities in localized areas (Ma *et al.* 1993).

EFB is composed of 45-50% cellulose and about equal amounts (25-35%) of hemicellulose and lignin. It is fibrous, and the fibers stick together to form vascular bundles. There is a growing interest in composting EFB, in order to add value, and also to reduce the volume to make application easier, so that pretreatment comes into action to make it happens. Pretreatment on EFB is the first step required to fractionate lignocellulosic materials into its major plant components of lignin, cellulose and hemicellulose. An important goal of pretreatment is to increase the surface area of lignocellulosic material, making the polysaccharides more susceptible to hydrolysis. Along with an increase in surface area, pretreatment effectiveness and hydrolysis improvement has been correlated with removal of hemicellulose and lignin and the reduction of cellulose cyrstallinity. The large number of pretreatments used for lignocellulosic materials can be classified into groups as physical, physico-chemical, chemical, and biological processes. In this study physical pretreatment methods will also applied including mechanical comminution. Mechanical comminution is simply breaking the biomass into smaller pieces mechanically. This can include grinding, chipping and milling.

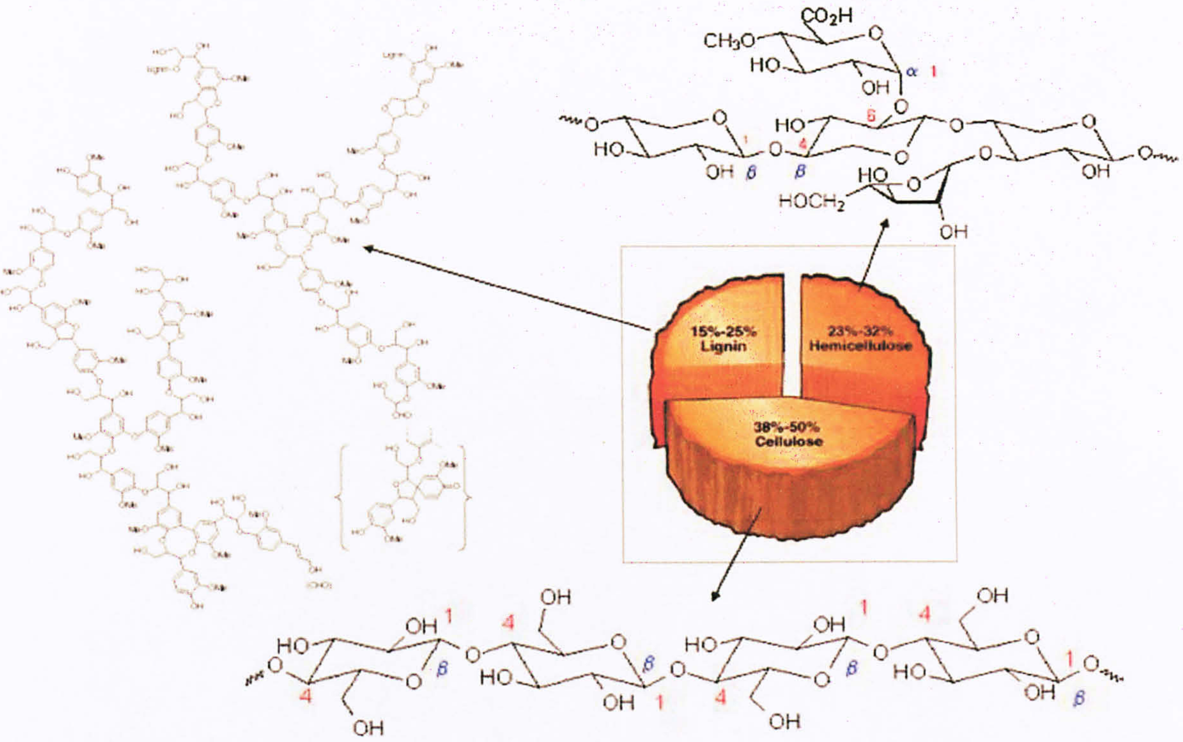


Figure 1.1: Biomass Compositions

1.2 Problem statement

There has been a renewed interest worldwide in biomass as energy source especially in producing bio-hydrogen. EFB is one of the municipal solid waste (MSW) and suitable as biomass feedstock for pre-treatment prior to alkaline/base of lignocellulosic to undergo gasification process which is favorable in producing bio-hydrogen compared to other process that available and the feedstock of the process must meet certain criteria like certain shape and small particle size. The main effect of alkaline/base pre-treatment on lignocellulosic biomass is delignification by breaking the ester bonds cross-linking lignin, thus increasing the porosity due to swelling allowed by breaking of the cross-links to be suitable for gasification feedstock. But the challenge of using lignocellulosic waste as the biomass resource is it would not easy to break lignocellulosic into smaller compound (sugar) as to get larger surface area for further process and use in producing bio-hydrogen.

1.3 Objective

The objectives of the research is to investigate the effect of alkaline/base pre-treatment using Sodium Hydroxide (NaOH) on the lignin degradation and the structure of empty fruit bunches (EFB) based on the concentration of total reducing sugars in hydrolysis process for bio-hydrogen production.

1.4 Scope of Work

To achieve high degradation of lignin which leads to high conversion of sugar from cellulose and hemicelluloses for EFB application to bio-fuel, bio-gas and bio-chemical industries especially in producing bio-hydrogen, pre-treatment process is required. The goal of pretreatment is to decrease the crystallinity of cellulose structure and increase the surface area of EBF by breaking down the polymer structure of lignocellulosic material into its corresponding monomer.

In this research, the focus is only on chemical pretreatment by using alkaline/base which is NaOH. But somehow, the physical pretreatment must be applied before proceed to other process like grinding the EFB to smaller sizes and sieving them into different sizes for experimental works. The experiments include the analytic techniques involved in improving capacity of lignin degradation of lignocellulosic in EFB. Then, an appropriate methodology was developed according to the step-by-step procedures from identifying, extracting, and analyzing the lignocellulosic. Analysis of the result obtained will be carried out to determine the effectiveness of alkaline/base used for the pretreatment process.

CHAPTER 2

LITERATURE REVIEW

2.1 Bioprocess for hydrogen production from biomass

To meet the expected increase in demand for hydrogen as fuel for fuel cells, renewable resources (wind, sun, hydropower, geothermics and biomass) will need to be used for hydrogen production in order to adhere to the Kyoto agreement. These technologies have all their specific advantages and disadvantages in terms of potential, efficiency, scale and foreseen production cost. Most technologies for the production of renewable hydrogen are still in the R&D stage and world-wide subject of increased research efforts. At present, there are two strategies for the production of hydrogen from biomass: the thermochemical technology, such as gasification or supercritical water gasification, and the biological production technology using micro-organisms. Biological hydrogen production delivers clean hydrogen with an elegant and simple technology and is more suited for the conversion of wet biomass in small-scale applications as compared to the other thermochemical processes.

Biomass for hydrogen production ranges from domestic organic waste to more defined agro-industrial residues and finally to well-defined produce from energy crops. Several examples have been tested with respect to applicability for biological hydrogen production [Claassen *et al* 2002]. This far, most experiments have been successful, even with the limited number of thermophilic strains used. Lignocellulosic feedstock is generally favored because it is cheap and abundant. However, for obtaining high efficiencies, pretreatment for mobilization of fermentable sugars in hydrolysates is required (De Vrije *et al* 2002).

2.2 Gasification to Hydrogen Production

Hydrogen is recognized as one of the most promising energy carriers in the future. Many investigations on various hydrogen production methods have been conducted over the past several decades. Biomass is potentially a reliable energy resource for hydrogen production. Biomass is renewable, abundant and easy to use. The thermochemical pyrolysis and gasification hydrogen production methods are economically viable and will become competitive with the conventional natural gas reforming method.

2.2.1 Gasification Process

Gasification can be generally defines as a process for the production of gaseous or liquid fuels from organic material within the feedstock. Biomass can be gasified at high temperatures (above 1000 K). The biomass particles undergo partial oxidation resulting in gas and charcoal production. The charcoal is finally reduced to form H2, CO, CO2 and CH4. This conversion process can be expressed as:

Biomass + heat + steam → H2 + CO + CO2 + CH4 + light and heavy hydrocarbons + char

Unlike pyrolysis, gasification of solid biomass is carried out in the presence of O_2. Besides, gasification aims to produce gaseous products while pyrolysis aims to produce bio-oils and charcoal. The gases produced can be steam reformed to produce hydrogen and this process can be further improved by water–gas shift reactions as discussed in the previous section. The gasification process is applicable to biomass having moisture content less than 35%, [A. Demirbas, 2002].

There are two fundamental stages in the gasification process:

- Organics in the feedstock are broken into compounds consisting largely of carbon and hydrogen
- These compounds then undergo reactions to form a liquid or gas with the significant amount of energy

Thermal processing remains a functional piece of the MSW (biomass like EFB) puzzle today because of its ability to reduce feedstock by an average of 90% in volume and 75% in weight and because the heat generated can be used to convert water into steam and thereby produce energy.

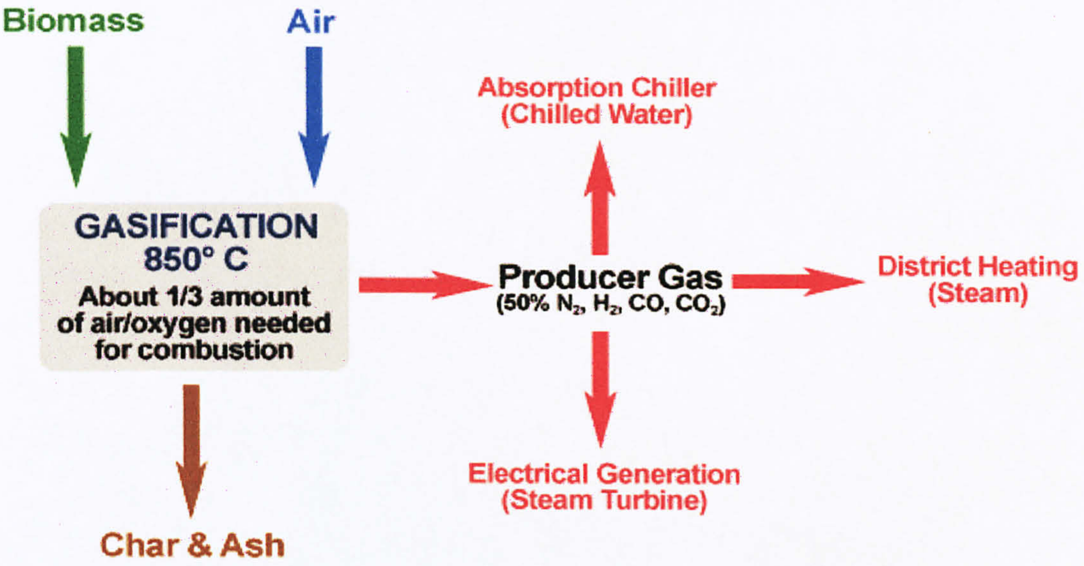


Figure 2.1: Biomass Gasification Process

2.2.2 Gasification Feedstock Characteristics

The secondary target of this study is to investigate on the effect of size, structure and shape towards gasification process by conducting the pretreatment process to produce hydrogen. The variable affecting the rate of gasification are to be identified and quantified from the pretreatment process of EFB using sodium hydroxide. The variables studied are given below:

1. Size

Smaller the biomass size better would be the heat transfer. The temperature would be uniform resulting in reaction taking place throughout the particle. Whenever reaction controls the gasification, the rate of reaction will be maximum and increases exponentially with temperature. However, there is a limitation on the size above which heat transfer would be controlling. [Maa and Bailie,1973] have shown that the pyrolysis of cellulose material is reaction controlled for size less than 0.2 cm, for sizes 0.2–6 cm both heat transfer and reaction control, whilst above 6 cm heat transfer controls.

2. Shape

Bio-residues are generally powder in nature. At present they are pelletized before gasification so as to reduce the volume of gasifier. Pelletization consumes power thus reducing the available energy. Lumps on the other hand can be gasified conveniently. However, lumps beyond 6 cm size are not suitable. In order to have uniform rate of gasification yielding uniform composition of producer gas, the lumps are cut into small chips having size less than 2.5 cm. Chipping is also an energy consuming operation.

3. Structure

If the biomass is highly porous, the surface area for reaction is very high and the diffusion of the reactant/product would be easy. Uniform temperature could be achieved throughout biomass resulting in continuous reaction at all portions of biomass yielding uniform composition of product gases. When the biomass is less porous, the temperature varies from the maximum at the exterior to the minimum at the interior. The reaction takes place only at the exterior surface. This surface shrinks with reaction. Because of the non-uniformity in temperature, drying, pyrolysis and gasification take place simultaneously yielding non-uniform composition of gases. [Grønli *et al.* 2002] have studied thermogravimetric analysis and devolatilization kinetics of hard woods such as alder, beech, birch and oak, and soft woods such as Douglas fir, pine A, pine B, redwood and spruce. They have observed that in the case of soft woods, the decomposition starts at lower temperature, the maximum temperature of weight loss for hemicellulose is higher and hemicellulose & cellulose zones are wider.

2.3 Alkaline/Base Pre-Treatment

On this study, alkalis pretreatment refer to the application of alkaline solutions such as NaOH, Ca(OH)2 (lime) or ammonia to remove lignin and a part of the hemicellulose, and efficiently increase the accessibility for gasification or torrefaction process. Size, shape and structure of the biomass as mentioned above would affect the gasification or torrefaction rate, therefore alkalis pretreatment comes into action. Smaller the biomass size better would be the heat transfer in the gasification. However, there is a limitation on the size above which heat transfer would be controlling. Beside, bio-residues are generally powder in nature. At present they are palletized before gasification so as to reduce the volume of gasifier. Structure of the biomass is also important because if the biomass is highly porous, the surface area for reaction is very high and the diffusion of the reactant/product would be easy and uniform temperature could be achieved throughout biomass resulting in continuous reaction at all portions of biomass yielding uniform composition of product gases. Alkalis pretreatment will take those effected variables into consideration for getting full conversion of biomass into gas in the gasification or torrefaction to produce bio-hydrogen.

The alkali pretreatment can result in a sharp increase in saccharification, with manifold yields [Kassim *et al.* 1986]. Pretreatment can be performed at low temperatures but with a relatively long time and high concentration of the base. However, alkaline pretreatment was shown to be more effective on agricultural residues than on wood materials. They found that sodium hydroxide pretreatment resulted in the highest level of delignification (65% with 2% NaOH in 90 min at 121°C) and cellulose conversion (60.8%). Compared with acid or oxidative reagents, alkali treatment appears to be the most effective method in breaking the ester bonds between lignin, hemicellulose and cellulose, and avoiding fragmentation of the hemicellulose polymers [Gaspar *et al.* 2007].

2.4 Effective Parameters in Pre-Treatment of Lignocelluloses

The inherent properties of native lignocellulosic materials make them resistant to hydrolyze. The aim of pretreatment is to change these properties in order to prepare the materials for gasification process. Since lignocellulosic materials are very complicated, their pretreatment is not simple either. The best method and conditions of pretreatment depend greatly on the type of lignocelluloses. The crystallinity of cellulose, its accessible surface area and protection by lignin and hemicellulose, degree of cellulose polymerization, and degree of acetylation of hemicelluloses are the main factors considered as affecting the rate of biological degradation of lignocelluloses [Wyman *et al.* 1996]. These factors will be discussed briefly below.

2.4.1 Crystallinity

The cellulose microfibrils have both crystalline and amorphous regions, and the crystallinity is given by the relative amounts of these two regions. The major part of cellulose (around 2/3 of the total cellulose) is in the crystalline form [Chum *et al.* 1985]. It is therefore expected that high-crystallinity cellulose will be more resistant to hydrolyze, and it is widely accepted that decreasing the crystallinity increases the digestibility of lignocelluloses [Fan *et al.* 1980]. Kim and Holtzapple [Kim *et al.* 2006] found that the degree of crystallinity of corn stover slightly increased from 43% to 60% through delignification with calcium hydroxide, which was related to removal of amorphous components (lignin, hemicellulose). This discussion may indicate that the crystallinity is an important factor in digestibility of lignocelluloses. However, it is not the only factor in effective hydrolysis of these materials, due to the heterogeneous nature of celluloses and the contribution of other components such as lignin and hemicellulose.

2.4.2 Effect of Accessible Surface Area

Several studies have shown a good correlation between the pore volume or population (accessible surface area for cellulase) and the chemical digestibility of lignocellulosic materials. The main reason for improvement in hydrolysis by removing lignin and hemicellulose is related to the cellulose accessible surface area. The effect of this area may correlate with crystallinity or lignin protection or hemicellulose presentation or all of them. Lignocellulosic materials have two different types of surface area: external and internal. The external surface area is related to the size and shape of the particles, while the internal surface area depends on the capillary structure of cellulosic fibers.

2.4.3 Effect of lignin

The cellulose and hemicellulose are cemented together by lignin. Lignin is responsible for integrity, structural rigidity, and prevention of swelling of lignocelluloses. Thus, lignin content and distribution constitute the most recognized factor which is responsible for recalcitrance of lignocellulosic materials to chemical degradation by limiting the alkalis accessibility; therefore the delignification processes can improve the rate and extent of chemical hydrolysis. However, in most delignification methods, part of the hemicellulose is also hydrolyzed, and hence the delignification does not show the sole effect of lignin [Wyman *et al.* 1996].Dissolved lignin due to e.g. pretreatment of lignocelluloses is also an inhibitor for cellulase, xylanase, and glucosidase. The composition and distribution of lignin might also be as important as the concentration of lignin. The reason for improved rate of hydrolysis by removal of lignin might be related to a better surface accessibility for alkalis by increasing the population of pores after removing of lignin.

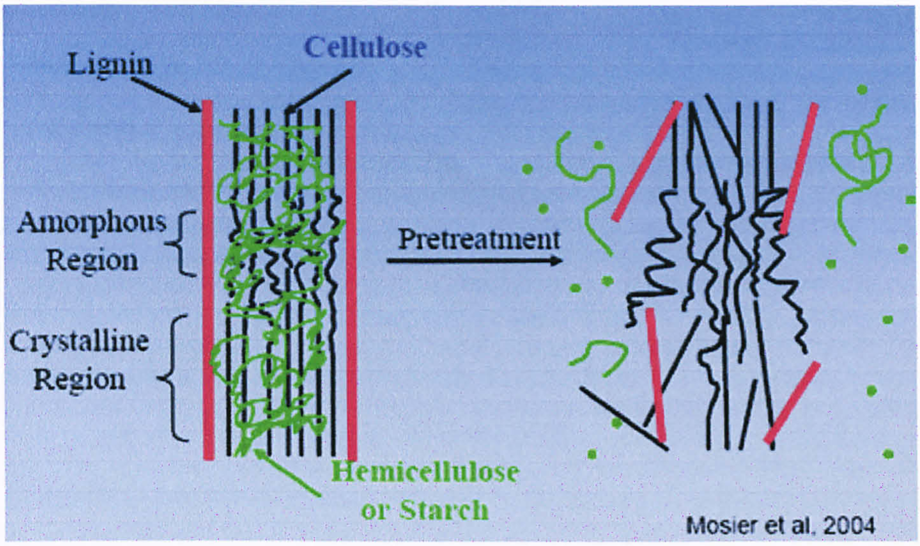


Figure 2.2: Schematic of goals of pretreatment on lignocellulosic material (Mosier *et al.*, 2004).

2.4.4 Effect of Hemicellulose

Hemicellulose is a physical barrier which surrounds the cellulose fibers and can protect the cellulose from alkalis attack. Many pretreatment methods were shown to be able to remove hemicelluloses and consequently improve the chemical hydrolysis. But most of these processes partly remove the lignin as well, so the improvement is not the result of removal of hemicellulose alone [Wyman e*t al.* 1996].The accessible surface for alkalis attack may be related to cellulose crystallinity, lignin, and hemicellulose content.

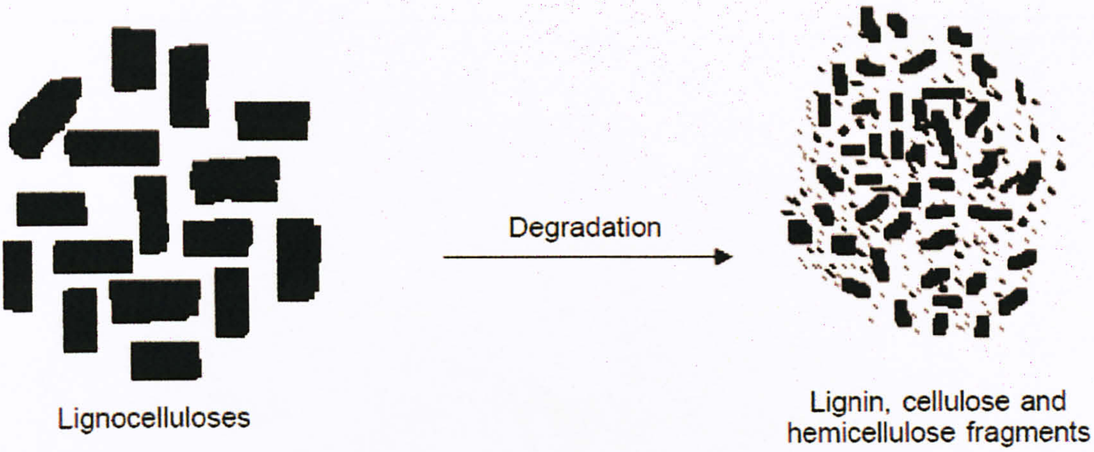


Figure 2.3: Pre-Treatment concept of lignocellulosic material

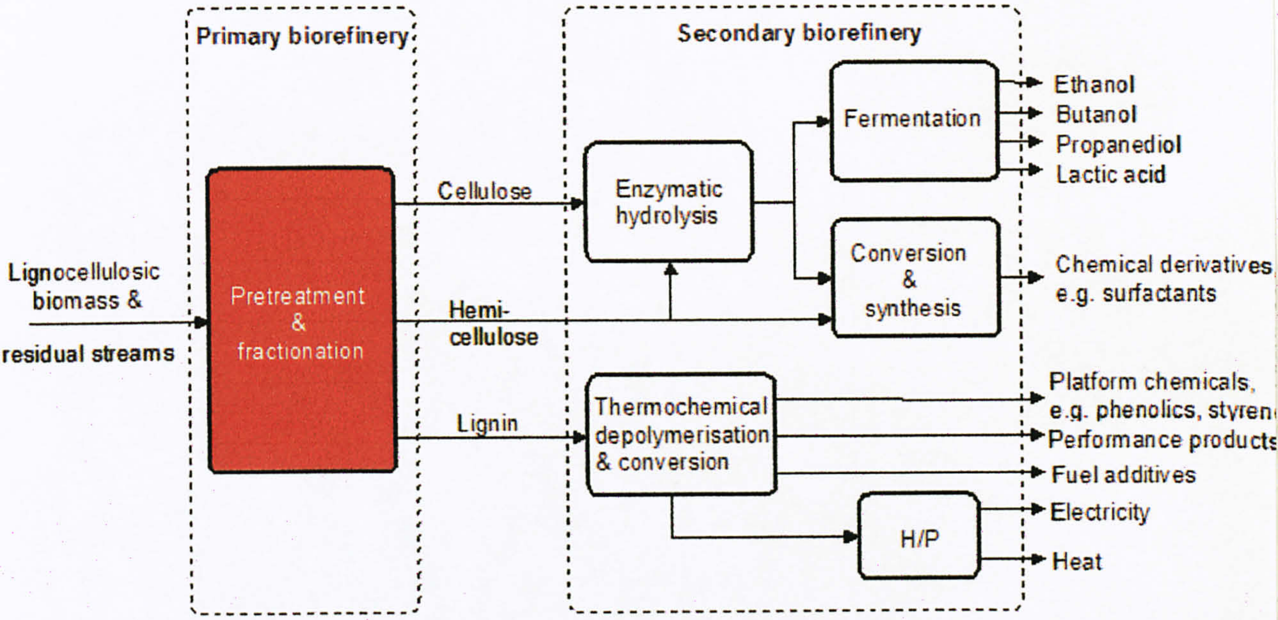


Figure 2.4: Possible bio-refinery including biomass fractionation

CHAPTER 3

MATERIAL AND METHOD

The focus of this project was to measure the lignin digestibility and crystallinity before and after the alkaline pre-treatment by determining the amount of sugars released from acid hydrolysis process.

Table 3.1: Main characteristic of EFB (%) [Ani, F. N., 2001]

Proximate analysis (%)	
Cellulose	59.7
Hemicellulose	22.1
Lignin	18.1
Ash	5.36
Elemental analysis	
Carbon	47.89
Hydrogen	6.05
Nitrogen	0.65
Oxygen	45.41
Empirical formula	$H_{1.51}N_{0.01}O_{0.95}$
H/C molar ratio	0.1263
O/C molar ratio	0.9482
N/C molar ratio	0.0136
High heating value (MJ/kg)	16.7405

3.1 Preparation of Biomass Material (EFB)

Palm Oil EFB obtains from FELCRA palm oil milling plant in Seri Iskandar, Perak. Initially, the fiber will be washed and make them dust free. Then, it will be dried in a drying cabinet at 100°C for about 24 hours. Physical pre-treatment was carried out using grinding machine in the laboratory in order to achieve a large surface area of EFB for reaction with sodium hydroxide. Siever is used to get different size of samples ranging from1000μm to 125μm as shown in **Figure**

3.1.The size of the solid was suggested to be between 0.5-1 mm. Water and oil content was initialized at the preliminary analysis using Sokhlet method.

(a) (b)

(c)

Figure 3.1: (a) Dried EFB (b) Sieving (c) EFB in different particle sizes

3.2 Sodium Hydroxide Pre-Treatment Procedure

EFB ground and sieved to 125µm, 250µm and 500µm were pre-treated with 20, 40 and 60%, NaOH. Each pre-treatment is done at 5g solids loading and each is put into a 250 Erlenmeyer flask. 100 ml of chemical solution is added to the Erlenmeyer flask and placed in a temperature controlled water bath shaker with agitation speed of 100 rpm at temperature of 45°C for 75 minutes. Then sample from the flask is taken out and cooled in water bath to about 10°C in 10 minutes. The hydrolyzate was collected and filtered through filter paper, Whatman #1. Then the filter cake was washed several times with distillated water until the pH of the filtrate was 7. The resulting cake was dried at the temperature of 50°C for 24 hours and they were used for total sugars analysis in hydrolysis process. Lignin analysis was completed by using UV-Vis Spectrophotometer for determining the concentration of total sugars released and using Scanning Electron Microscope (SEM) for analyzing a morphology/structure of the samples after the samples were dry.


(a) (b)

Figure 3.2: (a) 500µm untreated EFB and (b) 500µm treated EFB using 20% NaOH

3.3 Preparation of DNS Reagent

The DNS procedure used was a modified version of LAP-006 from NREL [Adney & Baker, 1996]. LAP-006 is a procedure for cellulase enzyme activity so only the parts relevant to DNS assay itself were referenced. First, the DNS Reagent was prepared by mixing 400mL of distilled water with 10.0g of 3,5-Dinitrosalicylic acid and 20.75 mL of 19.3N (50% w/w) sodium hydroxide. Three hundred grams of Rochelle salts (sodium potassium tartrate tetrahydrate), 7.5 ml Phenol (melted at 60°C) and 8.3g Sodium metabisulfate were thwn added and dissolved. Deionized water was added up to 1 L. The solution was stored in the dark by wrapping the bottle with aluminum foil. However, all chemicals except 3, 5-dinitrosalicyclic acid were not available in the laboratory which make the DNS Reagent cannot be prepared.

3.4 Preparation of Standard Curve of Glucose

0.5 gram of glucose was dissolved in a small quantity of distilled water and the volume was raised up to 100 ml. The stock solution (5.0 mg/ml) was used to make four appropriate glucose dilutions from 1g/ml – 5g/ml. Two millimeters of each dilution was taken in individual test tubes, 2.0 ml of 0.05 M 3, 5 DNSA and 8.0 ml of 2.5M NaOH was added into each tube. The tube was covered with parafilm and it shaken to mix thoroughly. The tubes were placed in a boiling water bath for 5 minutes. Next, they were removed from boiling water bath at proper time and quickly they were placed in an ice-water bath for 10 minutes. The % transmittance was measured at 546 nm on a UV-Vis spectrophotometer. A graph was plotted taking the transmittance at the coordinate and sugar concentration.

3.5 Hydrolysis Procedure for Determining Total Sugars Reducing

Batch experiments were conducted at 45°C using Sulfuric Acid (H_2SO_4) to catalyze the hydrolysis of cellulose. Treated solid of EFB from sodium hydroxide pre-treatment as stated above was charged into a 100ml flask containing 10% H_2SO_4, reaction time was set for 75 minutes. The flask was placed in a Water Bath Shaker with agitation speed of 150 rpm. The sample was withdrawn for sugar analysis at 15 minutes intervals. The define volume of the sample was immediately naturalized to stop the reaction. Then the diluted sample was filtered through filter paper, Whatman#1. But, before analyzing the samples, the hydrolyzate was separated in the centrifuge to obtain pure liquid for testing. The total reducing sugar produced in the hydrolyzate was analyzed by the calorimetric method using UV-Vis spectrophotometer at 540nm using 3, 5-dinitrosalicycilc acid (DNSA) as a reducing chemical for glucose as stated above in the procedure of preparing the standard glucose curve (pure glucose was replaced by the treated samples from the hydrolysis process).

3.6 Determination of Sugar as Glucose

However, as mentioned before, all chemicals except 3, 5-dinitrosalicyclic acid were not available in the laboratory which make the DNS Reagent cannot be prepared. Therefore samples were only be added with 3, 5-dinitrosalicyclic acid and observation was made. The additional method is based upon the colour which forms when sugar reduces 3, 5-dinitrosalicyclic acid to 3-amino-5-nitrosalicyclic acid as shown in **Figure 3.1** and **Figure 3.2**. The pH should be in a basic solution, under which condition simpler sugar are good reduce agent.

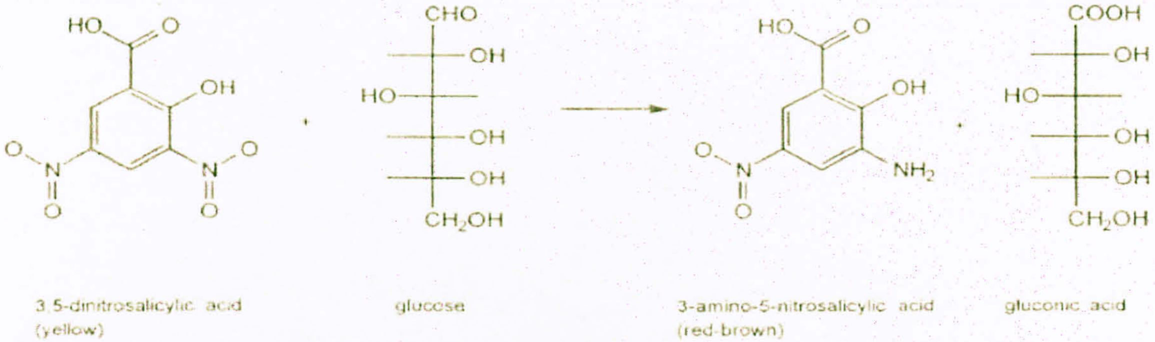
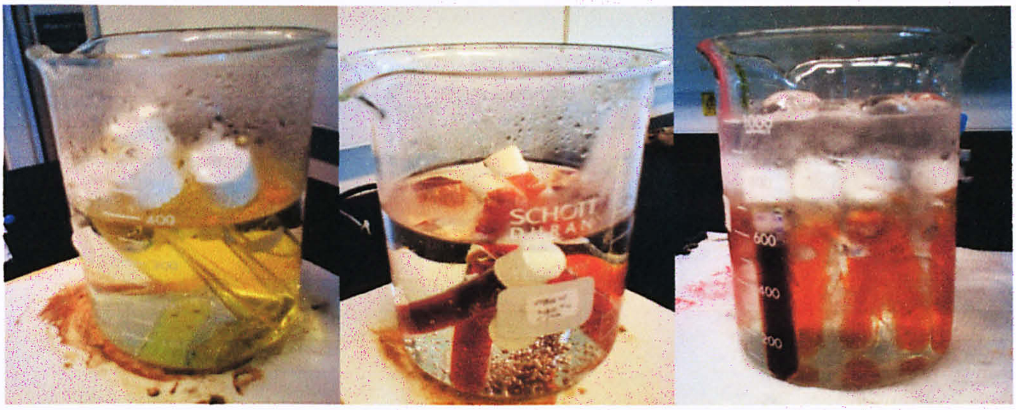


Figure 3.3: Sugar reduce 3, 5-dinitrosalicyclic acid (yellow) to 3-amino-5-nitrosalicyclic acid (red-brown)


(a) (b)

Figure 3.4: (a) Before reaction and (b) After reaction before the samples were taken out for total sugars analysis using UV-Vis spectrophotometer



(a) (b) (c)

Figure 3.5: (a) Once reaction started (heated), (b) 5 minutes after reaction started (heated) and (c) In an ice-water bath for 10 minutes immediately after heating up the samples.

CHAPTER 4

RESULTS AND DISCUSSION

Alkaline/base pre-treatment of untreated EFB and acid hydrolysis of pretreated EFB of palm oil residue was carried out in several sets of experiments. Batch experiments were conducted using sodium hydroxide (NaOH) and acid sulfuric (H_2SO_4) as the reaction catalyst. For maximum capacity of lignin degradation in pre-treatment process, only 2 parameters were considered such as solid particle size and alkaline/base concentration with respect to the liberated sugars in hydrolysis process using acid. Each parameter was evaluated and analyzed to determine the optimum reaction condition for the maximum capacity of lignin degradation.

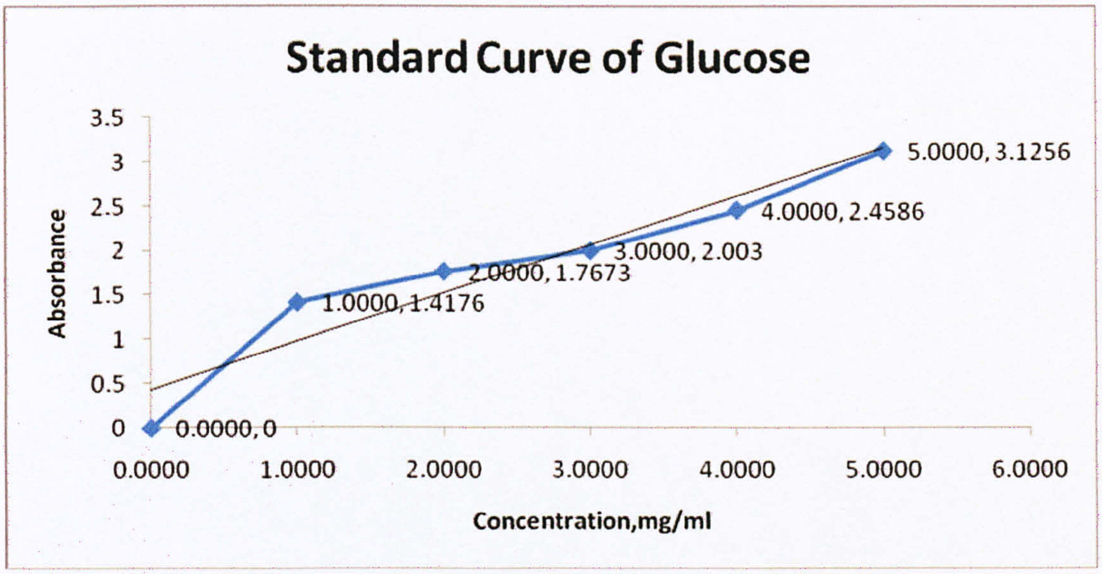


Figure 4.1: Standard Curve of Glucose

In this experiment, a standard curve of glucose will also be needed as a reference for glucose/total reducing sugars determination in each sample. Each sample tested was under laid within the range of the standard curve. In this experiment, a standard curve of glucose will also be needed as a reference for glucose/total reducing sugars determination in each sample. Each sample tested was under laid within the range of the standard curve. In spectrum analysis, wavelength range between 200 and 650 (nm) of was set when running the samples in UV-Vis Spectrophotometer and a peak obtained for each sample was overlaid at 319nm wavelength instead of expected wavelength of 540nm for red brownish colour for reducing sugars. Therefore, for a photometric analysis, wavelength of 319nm was set as a basis for the standard glucose and the samples analysis.

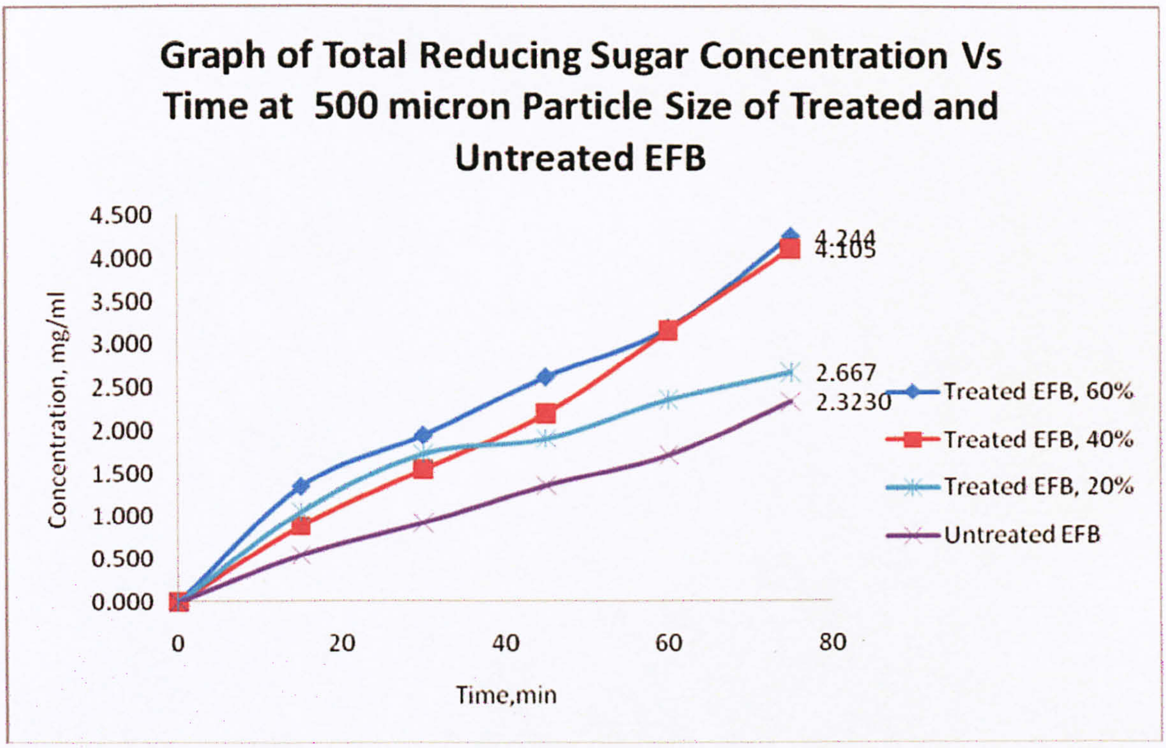


Figure 4.2: Total reducing sugar with respect to 500μm particle size of treated and untreated EFB fibers at 20% H_2SO_4, 5g solid at 45°C

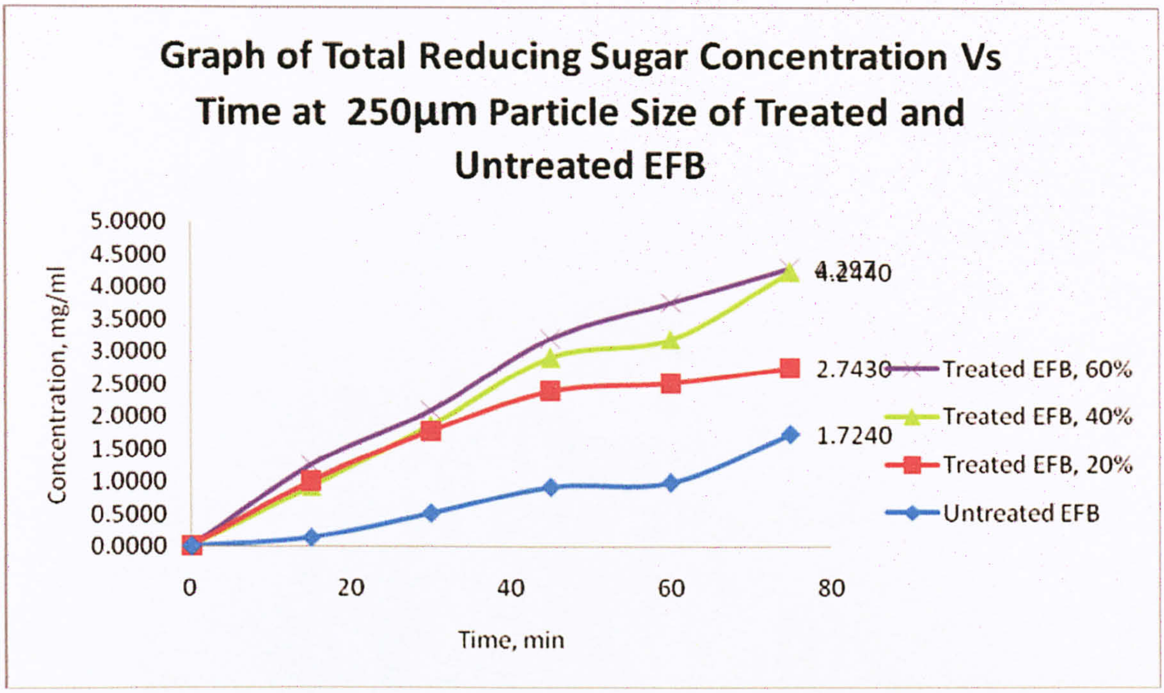


Figure 4.3: Total reducing sugar with respect to 250μm particle size of treated and untreated EFB fibers at 20% H_2SO_4, 5g solid at 45°C

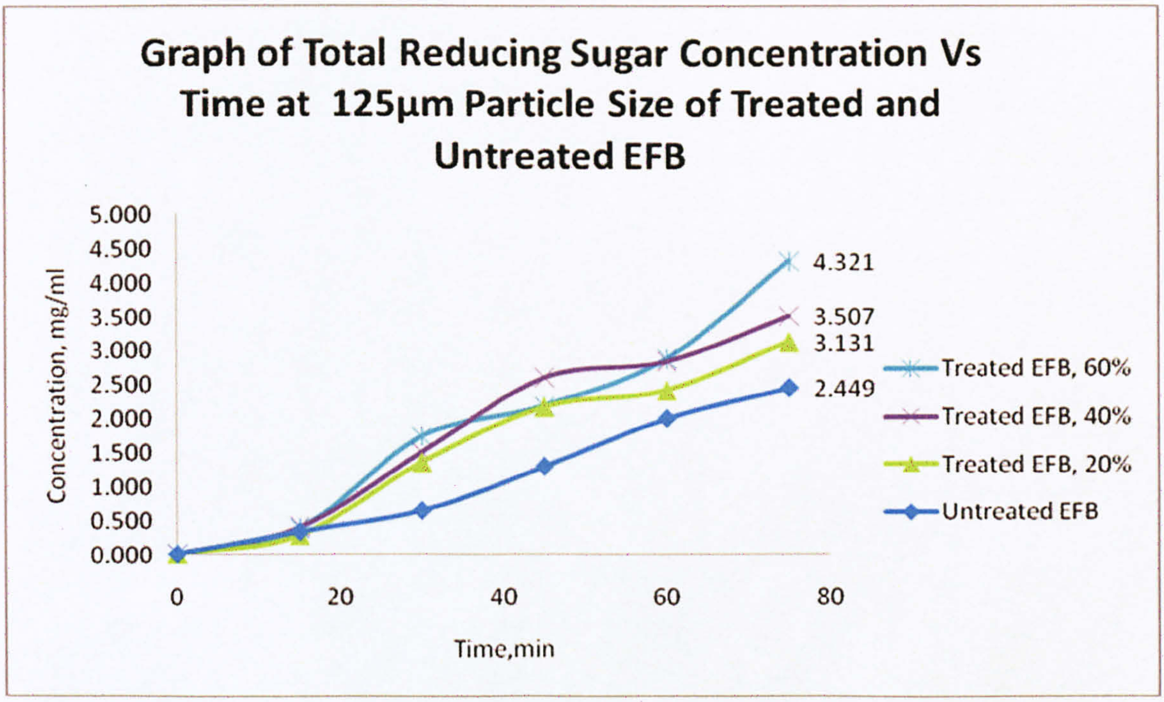


Figure 4.4: Total reducing sugar with respect to 125μm particle size of treated and untreated EFB fibers at 20% H_2SO_4, 5g solid at 45°C

4.1 The Effect of Lignin Degradation

Lignin is the most difficult component to be degraded due to its complex structure, high molecular weight and high insolubility. Through the pre-treatment process applied, it was observed that a significant reduction in lignin content has been achieved as compared to the original untreated substrate. All three set of batch experiments demonstrated different capacity in lignin degradation and the performance may be best described by the concentration of total reducing sugar as illustrated in the **Figure 4.2, Figure 4.3 and Figure 4.4.** The results demonstrated that the use of NaOH chemical in pretreatment of native EFB fibers gave encouraging performance in all related experiments conducted rather than the untreated EFB fibers with different particle sizes.

Concentrated base pretreatment of EFB fiber of 60% NaOH for each tested particle size showed more than 80% of conversion of cellulose to sugar which is 4.321mg/ml sugar concentration for 125μm particle size, 4.279mg/ml sugar concentration for 250μm and 4.242mg/ml sugar concentration for 500μm particle size. Untreated EFB fibers produced quite a small value of sugar concentration which only gave only 42% conversion of cellulose to sugar because in most lignocellulosic waste like EFB, due to the presence of cellulose crystallinity, the chemical like acid attack on the cellulose is retarded. Therefore, base pretreatment using NaOH was necessary to increase susceptibility of lignocelluloses for hydrolysis reaction. According to **Figure 4.4,** the impact of degradation seems to be more significant when smallest treated EFB fibers (higher concentration of NaOH used) were added in the reaction. The introduction of different sizes of treated EFB fibers in the **Figure 4.2 and Figure 4.3** have also shown remarkable improvement in lignin breakdowns based on the concentration of total reduced sugar produced in the hydrolysis process. Small particles have provided more surfaces for alkaline to contact and break the structure of the solid and alkaline penetrated faster into the small solid particles. In addition, longer reaction time was necessary if large solid sizes were used. According to [Rebecca et.al, 2007], an increase of NaOH concentration significantly improved lignin degradation capacity when temperature and residence time are combine.

Furthermore the cellulose fraction swelled and transformed from its crystalline form the more reactive amorphous form [Abdul Aziz., 2002]. In addition it was also understood that the lignin might be removed after NaOH treatment leaving carbohydrate fraction reactive towards further acid hydrolysis. In addition with the pre-treatment of EFB fibers with higher concentration of NaOH, the yield of total sugar reduced sugar was observed to increase the sugar concentration with different particle sizes of treated and untreated EFB. This may be attributing by the solubilization and swelling of hemicelluloses and cellulose fraction respectively and exposed the EFB fiber towards the catalytic reagent. In the pre-treated EFB with NaOH solution, the sequential actions that involved will be:

- The hemicelluloses solubilization
- The increase of cellulose and hemicelluloses digestion activity
- The improve of cellulose and digestion rate

In general, alkaline/base pre-treatment has been widely established for the purpose of alteration of biomass materials for pulp Kraft industries, lactic acid and bio-ethanol production [Rebecca et.al, 2007]. In this study, the technique was manipulated to alter the lignocellulosic structure of EPFB for the intention to produce chemicals. The pre-treatment is primarily employed to degrade the lignin structure. The superiority of the pre-treatment seems comparable to other pre-treatment methods as reported in other studies tabulated in Table 3. The use of NaOH for treating cotton stalk at 121°C was observed to give barely 66% of lignin degradation [Rebecca et.al, 2007]. An almost complete lignin degradation of EPFB was reported in this study. Interestingly, the pre-treatment was carried out under low temperature of 45°C. The low temperature signifies reduced energy consumption for the process.

4.2 Hydrolysis of Treated and Untreated EFB Fibers

Hydrolysis of EFB was carried out at concentration of acid 20% of H_2SO_4. Merely 48, 62, 70 and 86% conversion was achieved, respectively at reaction time of 75 minutes, 5g solid loading at 150 rpm in the Water Bath Shaker. As stated clearly by the numbers as shown in **Figure 4.1, Figure 4.2 and Figure 4.3**, the sugar concentration was increased with an increase in the alkaline/base concentration of treated EFB fibers that was applicable to the acid, catalyzed the hydrolysis process. The catalyst activity was proportional to H concentration. The more hydrogen ions formed in the solution, the more rapid the hydrolysis process occurred. Therefore the breaking of lignin bounds in the pre-treatment process will increase, causing high conversion of hemicelluloses fraction into xylose. But under harsh conditions of high acid concentration was applied, the cellulose fraction would be disrupted and glucose would be generated. In this study, xylose and glucose concentration is considered as total reducing sugars.

As acid acts as a catalyst, high concentration of acid may speed up the reaction rate while improving the sugar concentration. The experiment was conducted at 45°C with an acid concentration only 20% of H_2SO_4. **Figure 4.2, Figure 4.3 and Figure 4.4** shows the reduced sugar liberated from acid hydrolysis of 5g solid loading of treated and untreated EFB fibers at 45°C. The higher sugar concentration was associated with high alkaline/base concentration for 60% of NaOH for each particle size of treated EFB fibers and the lowest reduced sugar concentration resulted in each particle size of untreated EFB fibers. Experiments with very fine particle may possibly cause by the presence of impurities such ash from the grinding process. Hydrolysis of EFB with a solid size of 500μm resulted in acceptable glucose concentration and the liberated glucose concentration was increased with reaction time It was observed that a rapid reaction time between 40 to 75 minutes was adequate for maximum sugar concentration.

However, the sugar concentration decreased at high acid concentration at elevated temperatures, the longer reaction time at high temperature may cause deformation of sugar to furfural and hydroxyl methyl furfural which is un desired products originate from glucose and xylose decomposition at high temperature (greater than 55°C) in the presence of acid catalyst. Therefore, 20% of H_2SO_4 concentration at 45°C was chosen as concerned to obtain the total reducing sugar for treated and untreated EFB fibers with different particle sizes towards the capacity of lignin degradation in pretreatment process.

4.3 Structure of Treated and Untreated EFB Fibers

Because a large fraction of hemicelluloses and lignin was removed by pretreatment, there were some physical changes in the EFB fibers. The SEM photograph of untreated EFB fibers and treated EFB fibers were displayed in **Figure 4.4** and **Figure 4.5**. Effect of sodium hydroxide on the pretreatment study was done using different base concentrations from 20% to 60% of sodium hydroxide. Sodium hydroxide was applied for the pretreatment of 500µm, 250µm and 125µm EFB. According to the results obtained, the shape and the surface area of EFB fibers subjected to the base treatment differed significantly compared with the untreated EFB. The untreated EFB fibers or native fibers exhibited rigid and highly ordered fibrils, while the fibers of pretreated samples by sodium hydroxide were distorted. As reported by [Xu et al., 2007), the microfibril of the fibers were separated from the initial connected and fully exposed in the treatment, thus increasing the external surface area and the porosity. The native EFB fibers appeared as a rigid structure with well organized of silica embedded on the EFB's surface. Any crack or rupture structure was not noticed on the surface of the untreated EFB which indicated that the cellulose content inside the untreated EFB was properly hindered by lignin and hemicelluloses structure.

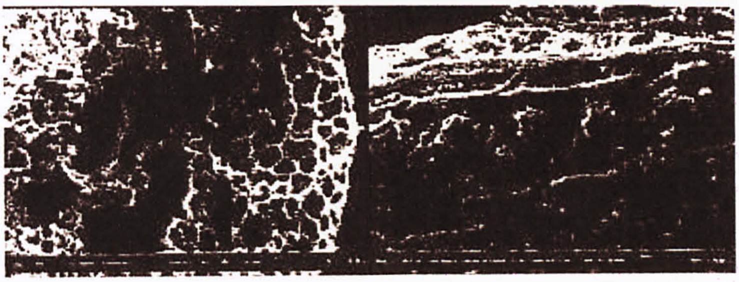
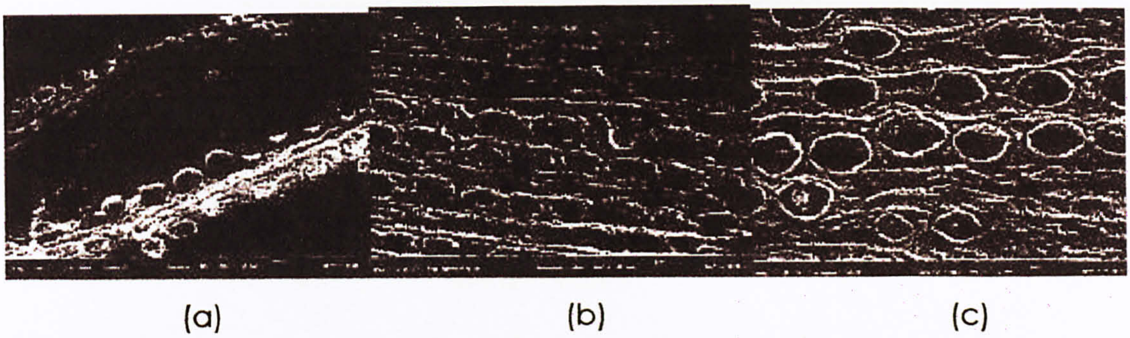


Figure 4.4: SEM photograph of native EFB (untreated)



(a) (b) (c)

Figure 4.5: SEM photograph of 500µm treated EFB using different concentration of NaOH, (a) 20%, (b) 40%, and (c) 60%

With the increase in the NaOH concentration, the fibers firther becomes smaller and thinner compared with the native EFB when the fibers were subjected to 20% NaOH, the surface of the fibers started to disrupt and changes in the EFB fibre surface is required in order to make the fibre's accessible to attack by the hydrogen ions [Shujun et al., 2007]. Beside converting lignin to its more soluble sodium derivative, sodium hydroxide used in pre-treatment also remove high molecular weight carbohydrate component from the cell wall structure, thereby causing an enlargement of the pores in the lignocellulose substrate and an improvement in the accessibility to reagent. Similar results were observed when fibre was exposed to the 20, 40 and 60% NaOH. Most part of the treated fibers surface showed characteristic such as smooth and plastic like features. However due to the cost and purification step, the 20% was the appropriate concentration for the further application.

4.4 Crystallinity of the EFB Fibers

The chemical composition was not only the sole factor influencing the enzymatic hydrolysis. Physical properties and cellulose microstructure were among the potential factors influencing enzymatic hydrolysis. One frequently cited property was the crystallinity of the fibers. According to the stereochemistry of the cellulose molecule, hydroxyl groups from the interchain hydrogen bonds or interchain hydrogen bonds within continuous molecules, producing a semicrystalline polymer consisting of both crystalline and amorphous region component that are not accessible by water molecule. Intramolecular spacing of the crystalline region of the cellulose remains unaltered during the treatment. This is because the chains of the crystalline cellulose are orderly oriented in a three dimensional lattice whereas the chains of amorphous cellulose are randomly oriented.

CHAPTER 5

CONCLUSION

Pre-treatment of EFB by using sodium hydroxide (NaOH) has proven to be the suitable method in lignin degradation based on total reducing sugars in hydrolysis process as 62, 70 and 86% conversion of cellulose to sugars for treated EFB fibers and only 48% for untreated EFB fibers at reaction time of 75 minutes, 5g solid loading with 20, 40 and 60% of NaOH respectively. From the parameter studied the pre-treatment and hydrolysis of EFB present that better conversion was obtained with 125µm of particles compared to the other solid size. Sodium hydroxide used in the pretreatment of EFB fibers also results in the physical changes and reduction of crystallinity of the fibers. High concentration of sodium hydroxide results in the good pretreatment process. This is due to removal of lignin and hemicelluloses during the process. From various concentration of sodium hydroxide used, sodium hydroxide at 20% was an economical concentration for the treatment process. In addition, through the pre-treatment, the microfibrils of the fibers were separated from the initial connected structure and fully exposed to the hydrogen ion for hydrolysis process. Consequently, it increased the external surface area and the porosity of the fibers and suitable to be an effective feedstock for gasification process in producing bio-hydrogen. Higher degradation, shorter preparation period and lower temperature condition seem to make the pre-treatment method attractive for EFB application to bio-hydrogen.

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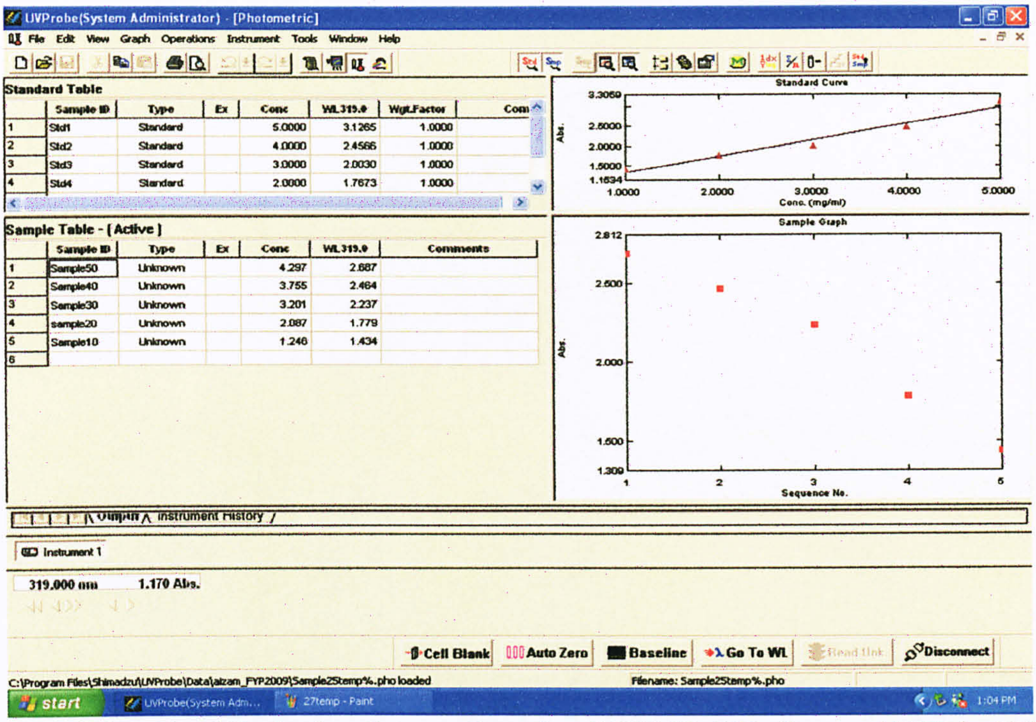
APPENDICES

Appendix 1: Experimental Data and Results from UV-Vis Spectrophotomer



Standard Table

	Sample ID	Type	Ex	Conc	WL319.0	Wgt.Factor	Com
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2	Std2	Standard		4.0000	2.4566	1.0000	
3	Std3	Standard		3.0000	2.0030	1.0000	
4	Std4	Standard		2.0000	1.7673	1.0000	

Sample Table - [Active]

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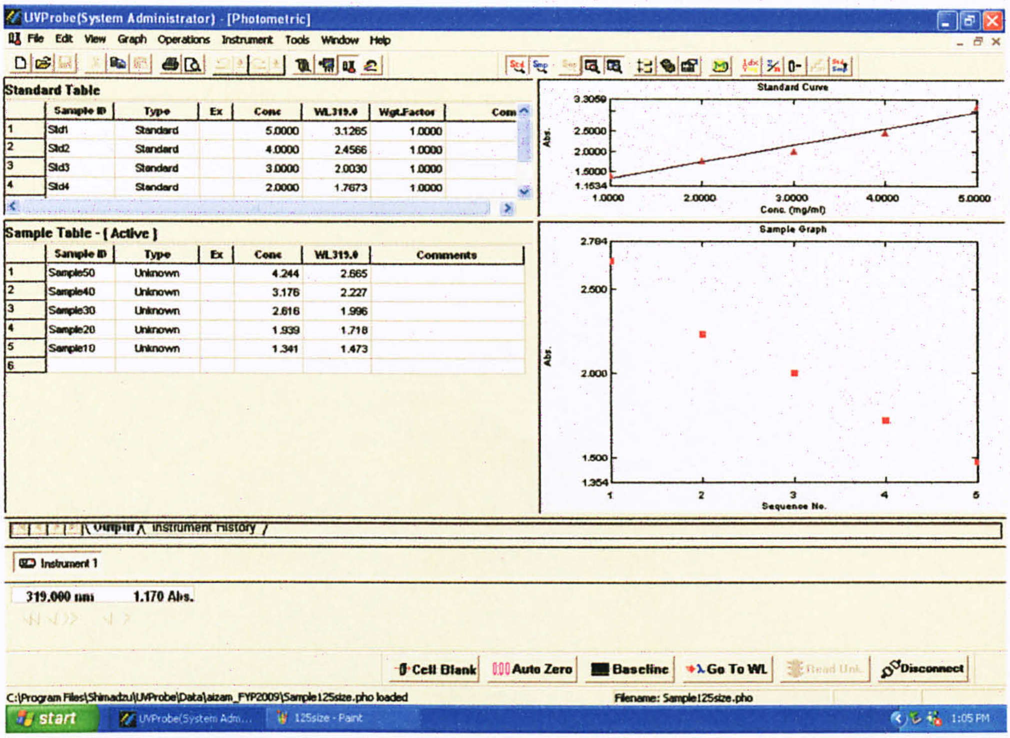
1	Sample50	Unknown		4.297	2.687	
2	Sample40	Unknown		3.755	2.464	
3	Sample30	Unknown		3.201	2.237	
4	sample20	Unknown		2.087	1.779	
5	Sample10	Unknown		1.246	1.434	
6						

319.000 nm 1.170 Abs.

C:\Program Files\Shimadzu\UVProbe\Data\aizam_FYP2009\Sample25temp%.pho loaded

Filename: Sample25temp%.pho

Figure AP1.1: Total Reducing Sugars: Sample of 60% NaOH of treated EFB in 250µm particle size



Standard Table

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2	Std2	Standard		4.0000	2.4566	1.0000	
3	Std3	Standard		3.0000	2.0030	1.0000	
4	Std4	Standard		2.0000	1.7673	1.0000	

Sample Table - (Active)

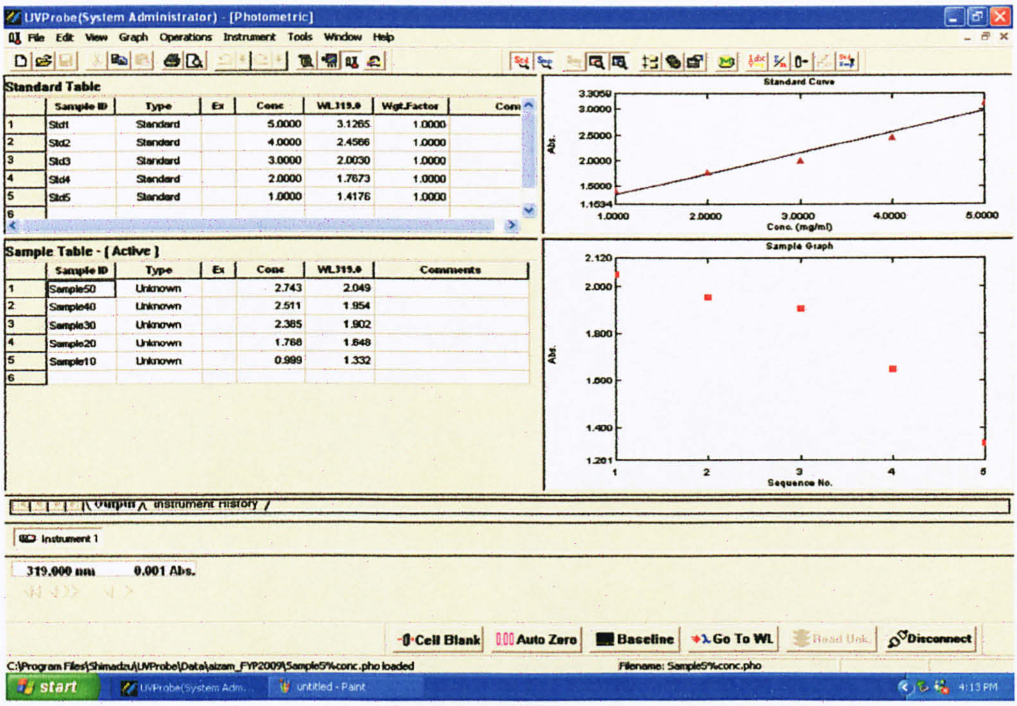
	Sample ID	Type	Ex	Conc	WL319.0	Comments
1	Sample50	Unknown		4.244	2.665	
2	Sample40	Unknown		3.176	2.227	
3	Sample30	Unknown		2.616	1.996	
4	Sample20	Unknown		1.939	1.718	
5	Sample10	Unknown		1.341	1.473	
6						

319.000 nm 1.170 Abs.

C:\Program Files\Shimadzu\UVProbe\Data\aizam_FYP2009\Sample125size.pho loaded

Filename: Sample125size.pho

Figure AP1.2: Total Reducing Sugars: Sample of 40% NaOH of treated EFB in 250μm particle size



Standard Table

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3	Std3	Standard		3.0000	2.0030	1.0000	
4	Std4	Standard		2.0000	1.7673	1.0000	
5	Std5	Standard		1.0000	1.4176	1.0000	
6							

Sample Table - (Active)

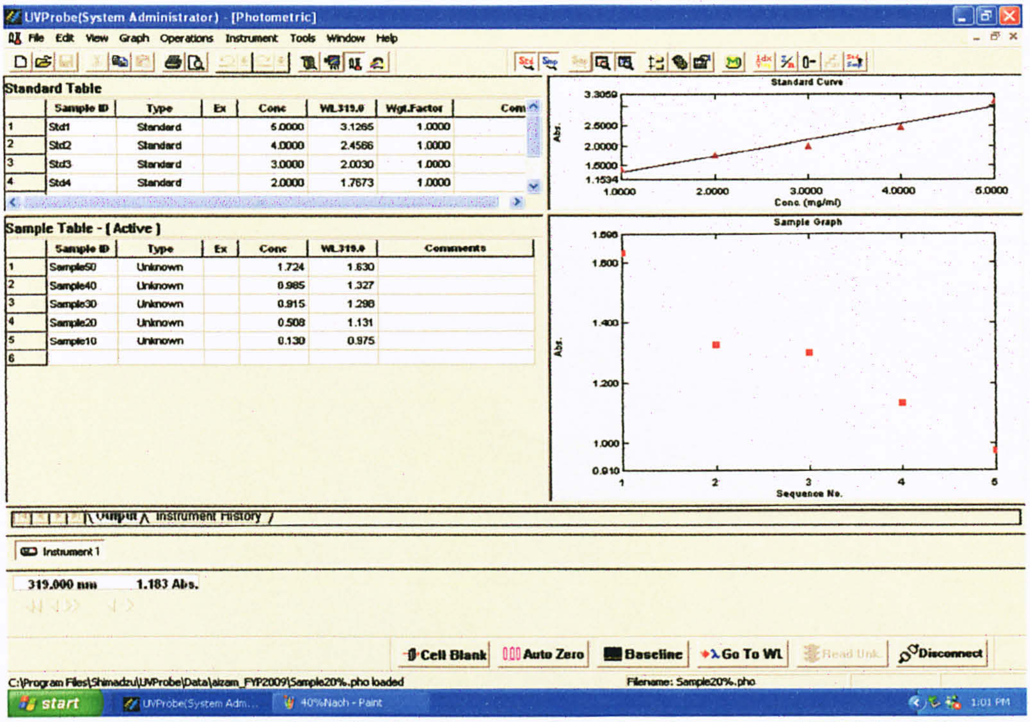
	Sample ID	Type	Ex	Conc	WL319.0	Comments
1	Sample50	Unknown		2.743	2.049	
2	Sample40	Unknown		2.511	1.954	
3	Sample30	Unknown		2.385	1.902	
4	Sample20	Unknown		1.768	1.648	
5	Sample10	Unknown		0.999	1.332	
6						

319.000 nm 0.001 Abs.

C:\Program Files\Shimadzu\UVProbe\Data\aizam_FYP2009\Sample5%conc.pho loaded

Filename: Sample5%conc.pho

Figure AP1.3: Total Reducing Sugars: Sample of 20% NaOH of treated EFB in 250μm particle size



Standard Table

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3	Std3	Standard		3.0000	2.0030	1.0000	
4	Std4	Standard		2.0000	1.7673	1.0000	

Sample Table - (Active)

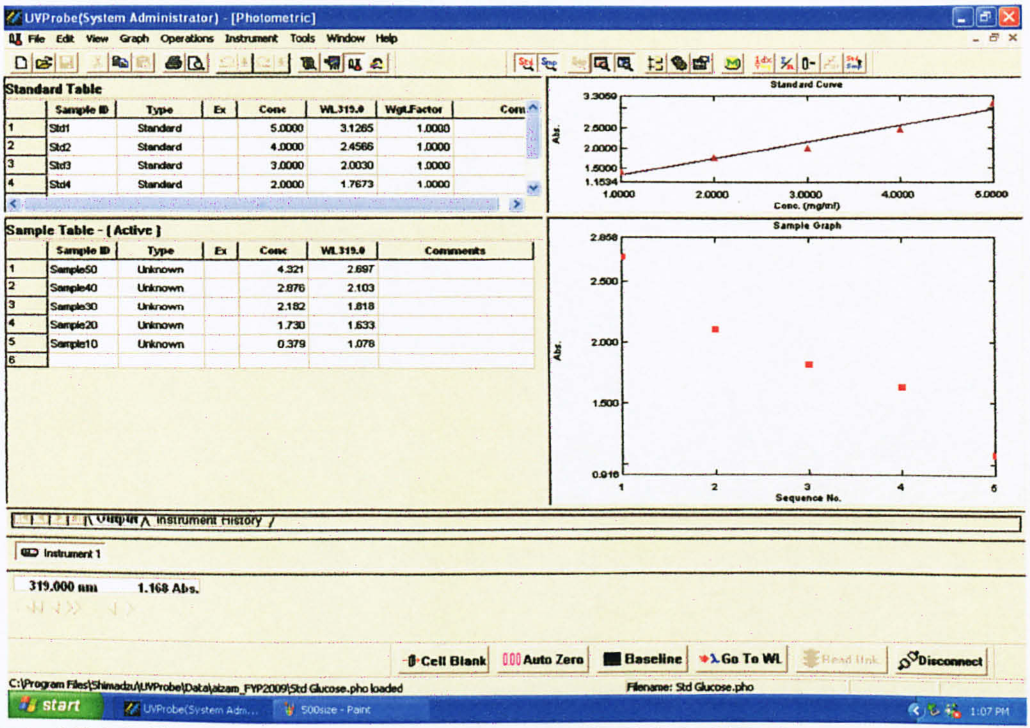
	Sample ID	Type	Ex	Conc	WL319.0	Comments
1	Sample50	Unknown		1.724	1.630	
2	Sample40	Unknown		0.985	1.327	
3	Sample30	Unknown		0.915	1.298	
4	Sample20	Unknown		0.508	1.131	
5	Sample10	Unknown		0.130	0.975	
6						

319.000 nm 1.183 Abs.

C:\Program Files\Shimadzu\UVProbe\Data\aizam_FYP2009\Sample20%.pho loaded

Filename: Sample20%.pho

Figure AP1.4: Total Reducing Sugars: Sample of untreated EFB in 250µm particle size



Standard Table

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3	Std3	Standard		3.0000	2.0030	1.0000	
4	Std4	Standard		2.0000	1.7673	1.0000	

Sample Table - (Active)

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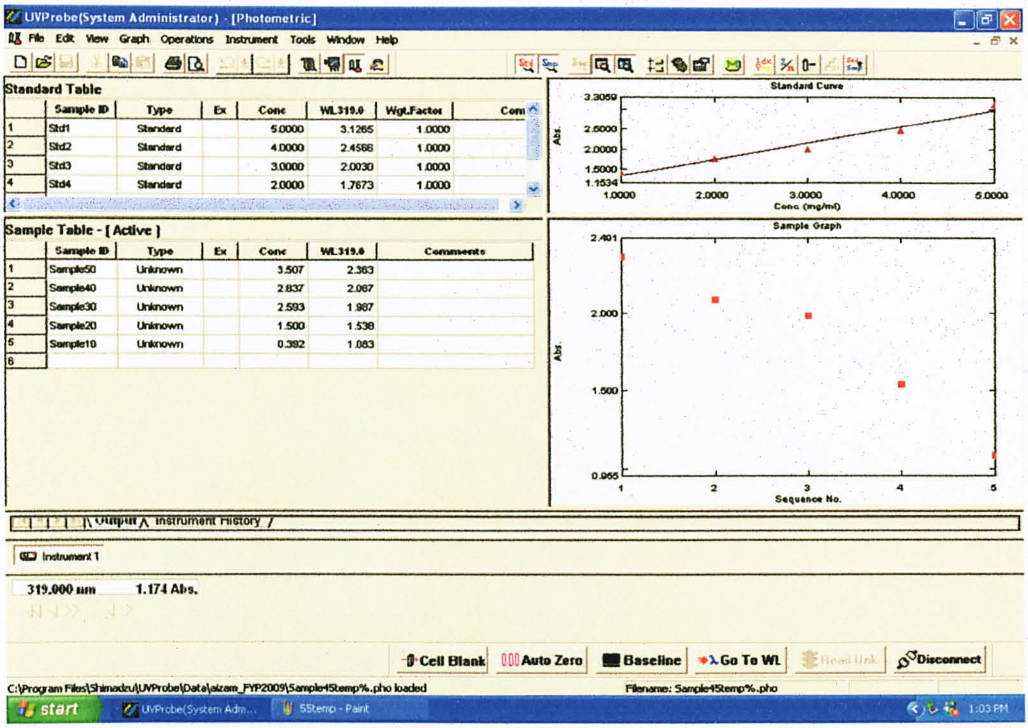
2	Sample40	Unknown		2.876	2.103	
3	Sample30	Unknown		2.182	1.818	
4	Sample20	Unknown		1.730	1.633	
5	Sample10	Unknown		0.379	1.078	
6						

319.000 nm 1.168 Abs.

C:\Program Files\Shimadzu\UVProbe\Data\aizam_FYP2009\Std Glucose.pho loaded

Filename: Std Glucose.pho

Figure AP1.5: Total Reducing Sugars: Sample of 60% NaOH of treated EFB in 125µm particle size



Standard Table

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2	Std2	Standard		4.0000	2.4566	1.0000	
3	Std3	Standard		3.0000	2.0030	1.0000	
4	Std4	Standard		2.0000	1.7673	1.0000	

Sample Table - [Active]

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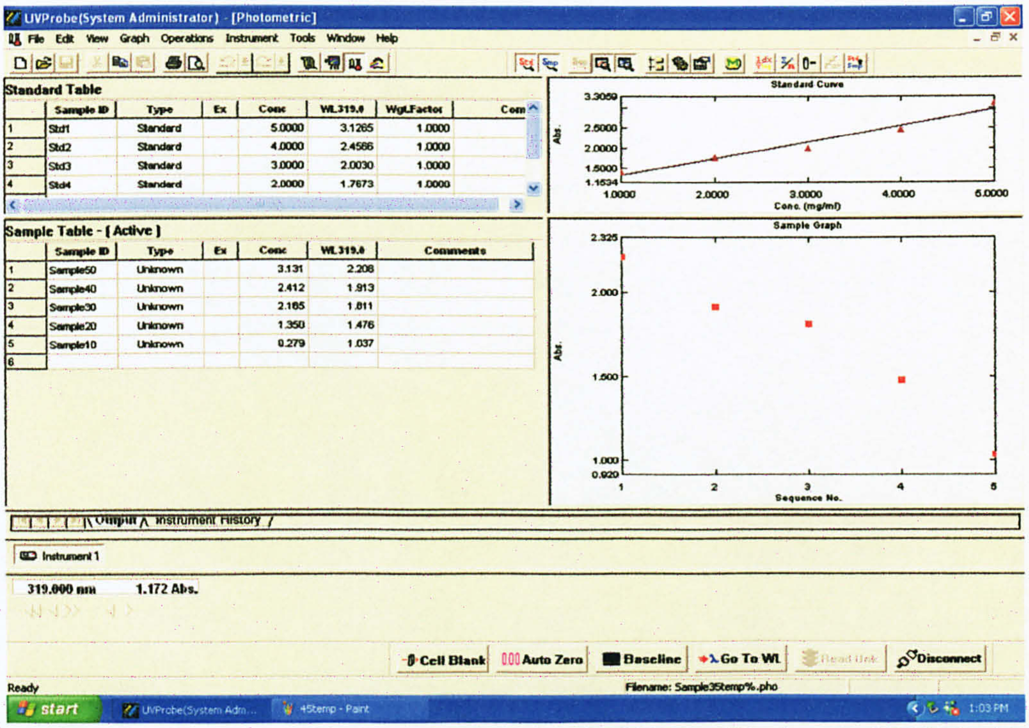
2	Sample40	Unknown		2.837	2.067	
3	Sample30	Unknown		2.593	1.987	
4	Sample20	Unknown		1.500	1.538	
5	Sample10	Unknown		0.392	1.083	
6						

319.000 nm 1.174 Abs.

C:\Program Files\Shimadzu\UVProbe\Data\aizam_FYP2009\Sample45temp%.pho loaded

Filename: Sample45temp%.pho

Figure AP1.6: Total Reducing Sugars: Sample of 40% NaOH of treated EFB in 125μm particle size



Standard Table

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3	Std3	Standard		3.0000	2.0030	1.0000	
4	Std4	Standard		2.0000	1.7673	1.0000	

Sample Table - [Active]

	Sample ID	Type	Ex	Conc	WL319.0	Comments
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2	Sample40	Unknown		2.412	1.913	
3	Sample30	Unknown		2.165	1.811	
4	Sample20	Unknown		1.350	1.476	
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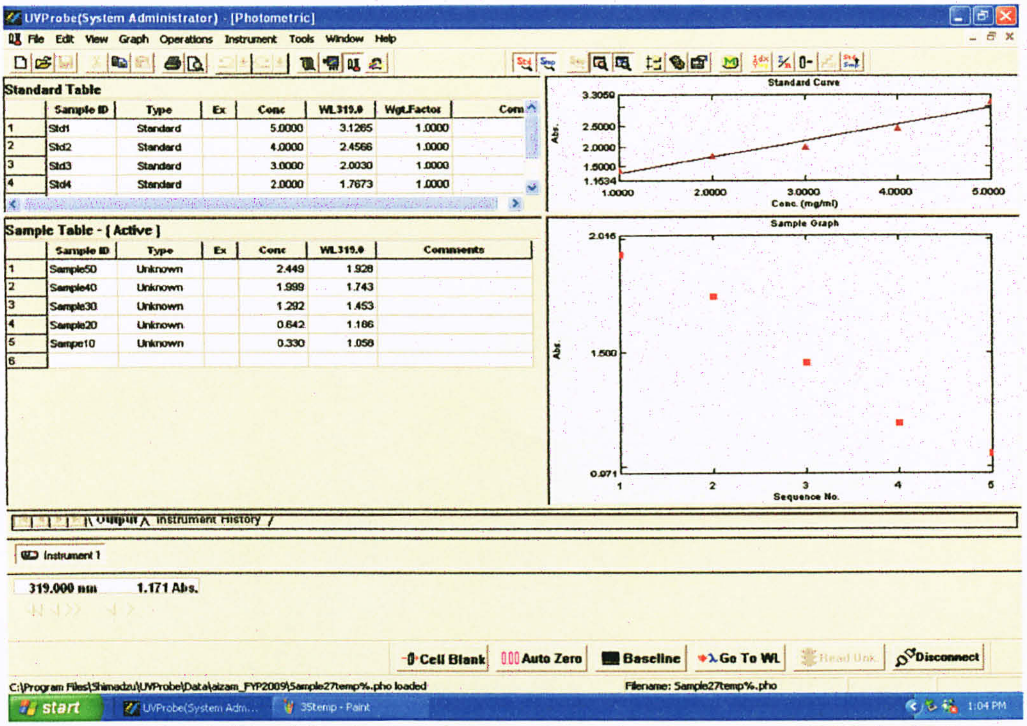
6						

319.000 nm 1.172 Abs.

Ready

Filename: Sample35temp%.pho

Figure AP1.7: Total Reducing Sugars: Sample of 20% NaOH of treated EFB in 125μm particle size



Standard Table

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2	Std2	Standard		4.0000	2.4566	1.0000	
3	Std3	Standard		3.0000	2.0030	1.0000	
4	Std4	Standard		2.0000	1.7673	1.0000	

Sample Table - (Active)

	Sample ID	Type	Ex	Conc	WL319.0	Comments
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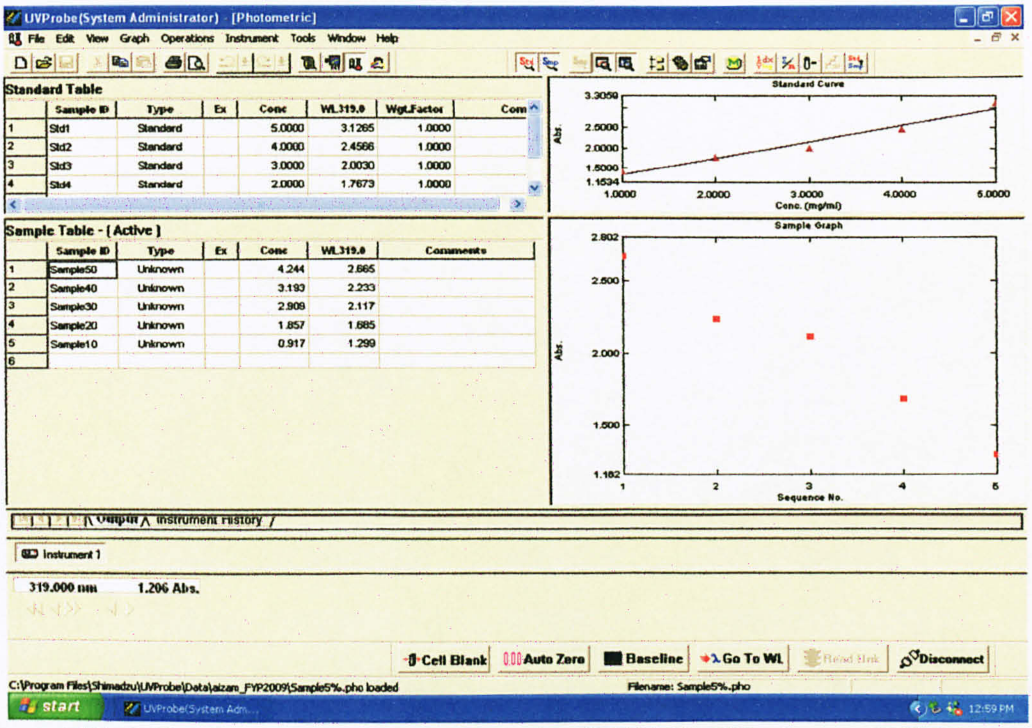
2	Sample40	Unknown		1.999	1.743	
3	Sample30	Unknown		1.292	1.453	
4	Sample20	Unknown		0.842	1.186	
5	Sampe10	Unknown		0.330	1.058	
6						

319.000 nm 1.171 Abs.

C:\Program Files\Shimadzu\UVProbe\Data\aizam_FYP2009\Sample27temp%.pho loaded

Filename: Sample27temp%.pho

Figure AP1.8: Total Reducing Sugars: Sample of untreated EFB in 125µm particle size



Standard Table

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2	Std2	Standard		4.0000	2.4566	1.0000	
3	Std3	Standard		3.0000	2.0030	1.0000	
4	Std4	Standard		2.0000	1.7673	1.0000	

Sample Table - (Active)

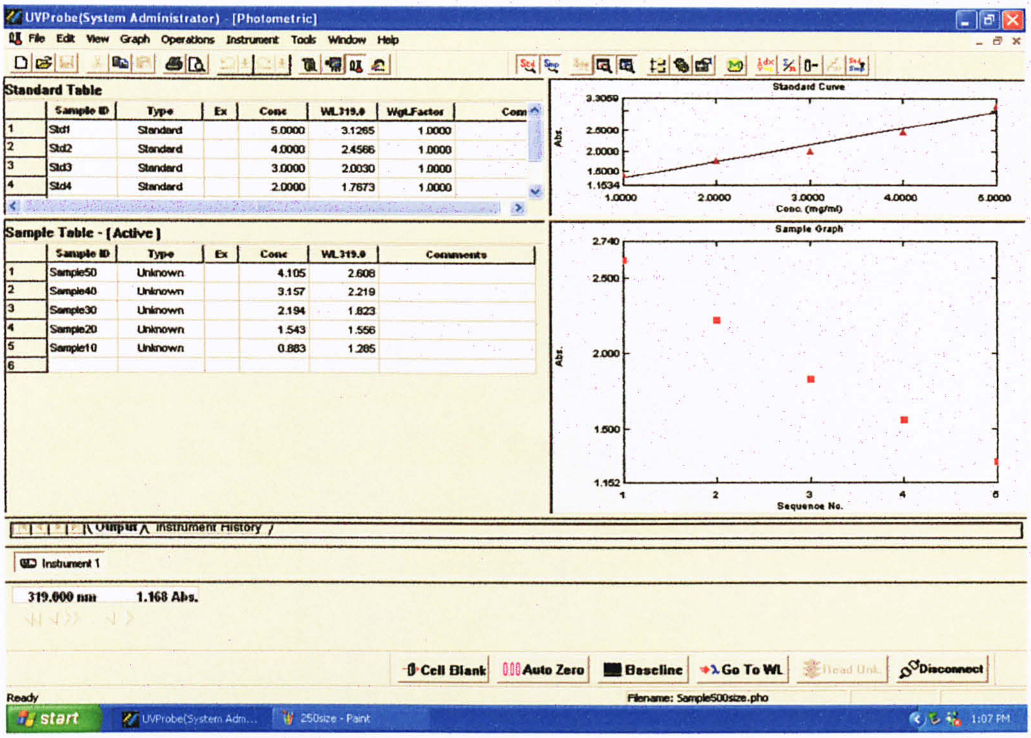
	Sample ID	Type	Ex	Conc	WL319.0	Comments
1	Sample50	Unknown		4.244	2.665	
2	Sample40	Unknown		3.193	2.233	
3	Sample30	Unknown		2.909	2.117	
4	Sample20	Unknown		1.857	1.685	
5	Sample10	Unknown		0.917	1.299	
6						

319.000 nm 1.206 Abs.

C:\Program Files\Shimadzu\UVProbe\Data\aizam_FYP2009\Sample5%.pho loaded

Filename: Sample5%.pho

Figure AP1.9: Total Reducing Sugars: Sample of 60% NaOH of treated EFB in 500µm particle size



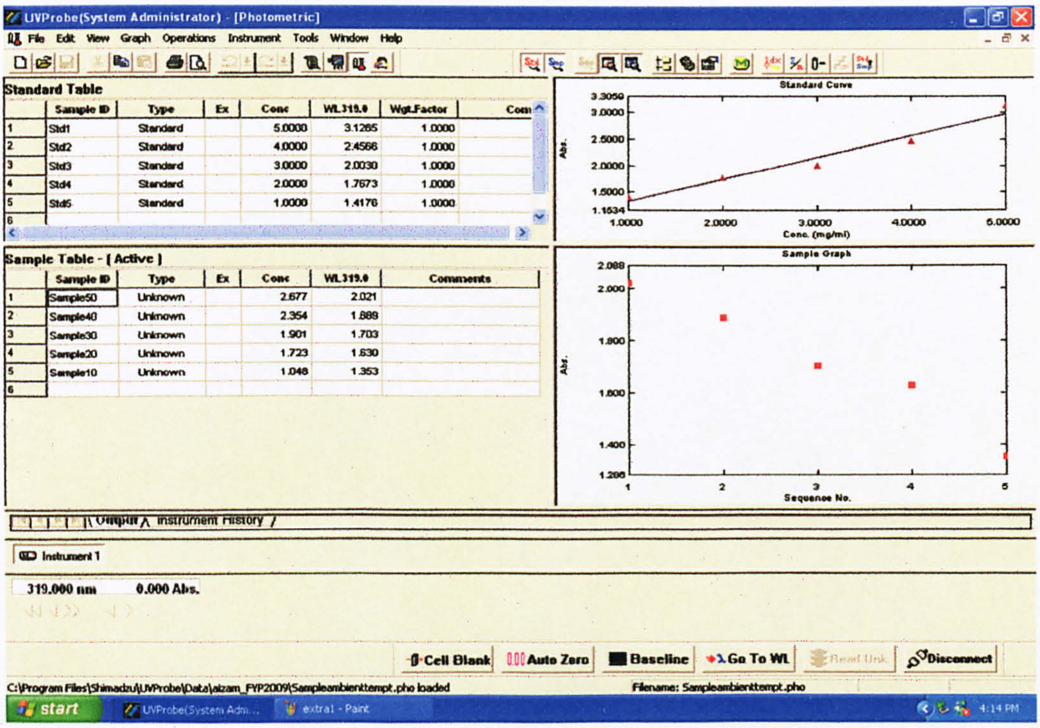
Standard Table

	Sample ID	Type	Ex	Conc	WL319.0	Wgt.Factor	Com
1	Std1	Standard		5.0000	3.1265	1.0000	
2	Std2	Standard		4.0000	2.4566	1.0000	
3	Std3	Standard		3.0000	2.0030	1.0000	
4	Std4	Standard		2.0000	1.7673	1.0000	

Sample Table - (Active)

	Sample ID	Type	Ex	Conc	WL319.0	Comments
1	Sample50	Unknown		4.105	2.608	
2	Sample40	Unknown		3.157	2.219	
3	Sample30	Unknown		2.194	1.823	
4	Sample20	Unknown		1.543	1.556	
5	Sample10	Unknown		0.883	1.285	
6						

Figure AP1.10: Total Reducing Sugars: Sample of 40% NaOH of treated EFB in 500μm particle size



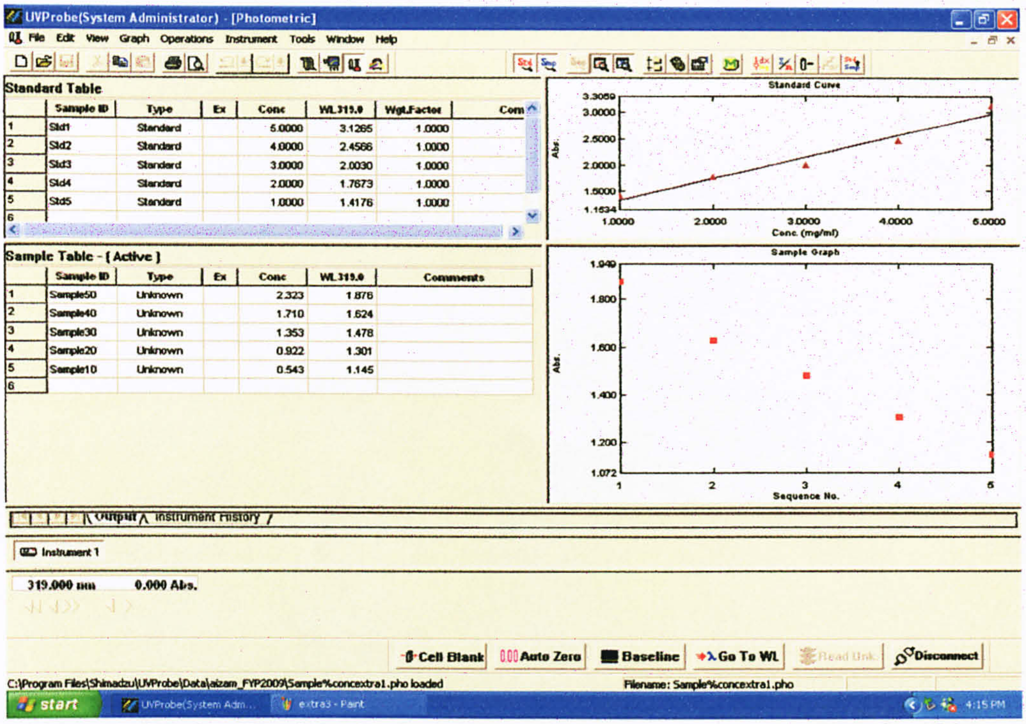
Standard Table

	Sample ID	Type	Ex	Conc	WL319.0	Wgt.Factor	Com
1	Std1	Standard		5.0000	3.1265	1.0000	
2	Std2	Standard		4.0000	2.4566	1.0000	
3	Std3	Standard		3.0000	2.0030	1.0000	
4	Std4	Standard		2.0000	1.7673	1.0000	
5	Std5	Standard		1.0000	1.4176	1.0000	
6							

Sample Table - (Active)

	Sample ID	Type	Ex	Conc	WL319.0	Comments
1	Sample50	Unknown		2.677	2.021	
2	Sample40	Unknown		2.354	1.889	
3	Sample30	Unknown		1.901	1.703	
4	Sample20	Unknown		1.723	1.630	
5	Sample10	Unknown		1.048	1.353	
6						

Figure AP1.11: Total Reducing Sugars: Sample of 20% NaOH of treated EFB in 500μm particle size



Standard Table

	Sample ID	Type	Ex	Conc	WL319.0	Wgt.Factor	Com
1	Std1	Standard		5.0000	3.1265	1.0000	
2	Std2	Standard		4.0000	2.4566	1.0000	
3	Std3	Standard		3.0000	2.0030	1.0000	
4	Std4	Standard		2.0000	1.7673	1.0000	
5	Std5	Standard		1.0000	1.4176	1.0000	
6							

Sample Table - [Active]

	Sample ID	Type	Ex	Conc	WL319.0	Comments
1	Sample50	Unknown		2.323	1.876	
2	Sample40	Unknown		1.710	1.624	
3	Sample30	Unknown		1.353	1.478	
4	Sample20	Unknown		0.922	1.301	
5	Sample10	Unknown		0.543	1.145	
6						

319.000 nm 0.000 Abs.

C:\Program Files\Shimadzu\UVProbe\Data\aizam_FYP2009\Sample%concextra1.pho loaded

Filename: Sample%concextra1.pho

Figure AP1.12: Total Reducing Sugars: Sample of untreated EFB in 500µm particle size

Appendix 2: Photographs of Equipments and Analyzers

Figure AP 2.1: Scanning Electron Microscopy (SEM)

Figure AP 2.2: UV-Vis Spectrophotometer

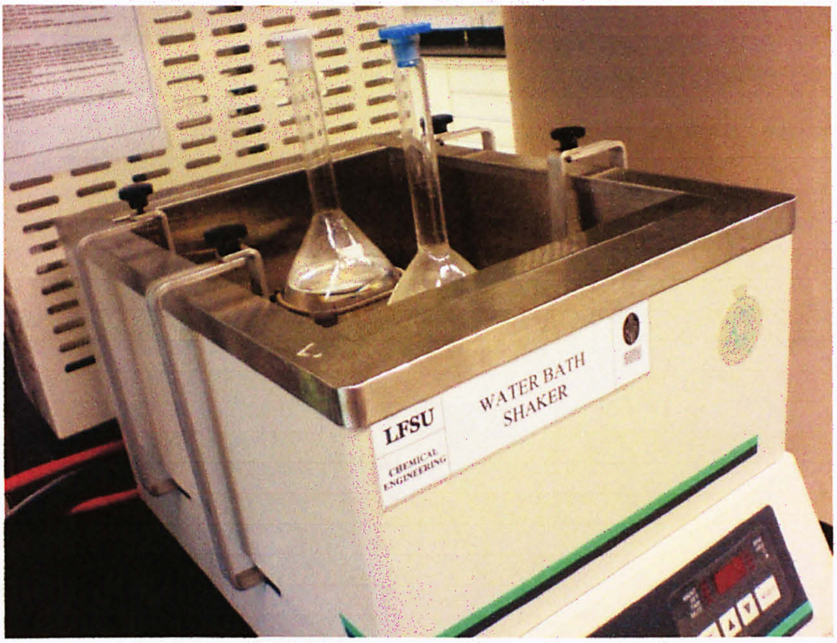
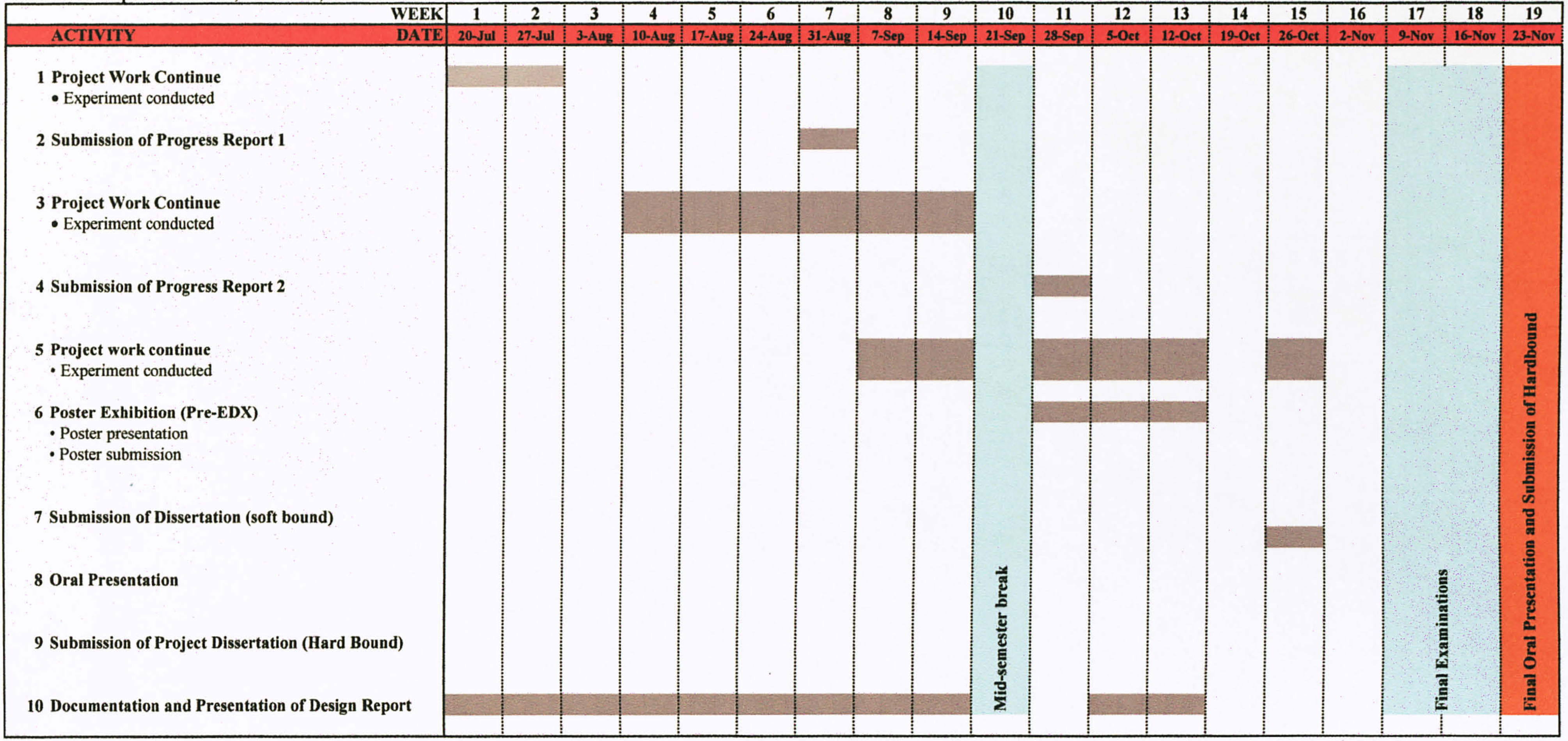


Figure AP 2.3: Reactor: Water Bath Shaker

Figure AP 2.4: Centrifuge Machine

FINAL YEAR PROJECT (FYP), July 2009 Semester: THE EFFECT OF BASE PRE-TREATMENT ON THE LIGNIN DEGRADATION AND STRUCTURE OF EMPTY FRUIT BUNCHES (EFB) FOR BIOHYDROGEN PRODUCTION

Timeline of Proposed Activities, Milestones, and Deadlines



WEEK	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
ACTIVITY DATE	20-Jul	27-Jul	3-Aug	10-Aug	17-Aug	24-Aug	31-Aug	7-Sep	14-Sep	21-Sep	28-Sep	5-Oct	12-Oct	19-Oct	26-Oct	2-Nov	9-Nov	16-Nov	23-Nov
1 Project Work Continue • Experiment conducted	▨	▨								Mid-semester break							Final Examinations		Final Oral Presentation and Submission of Hardbound
2 Submission of Progress Report 1							▨												
3 Project Work Continue • Experiment conducted				▨	▨	▨	▨	▨	▨										
4 Submission of Progress Report 2											▨								
5 Project work continue • Experiment conducted								▨	▨		▨	▨	▨		▨				
6 Poster Exhibition (Pre-EDX) • Poster presentation • Poster submission											▨	▨	▨						
7 Submission of Dissertation (soft bound)															▨				
8 Oral Presentation																			
9 Submission of Project Dissertation (Hard Bound)																			
10 Documentation and Presentation of Design Report	▨	▨	▨	▨	▨	▨	▨	▨	▨			▨	▨						

▨ Activities