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CHUNDLAG I NGINEBRING INIVERSIAT TEKNOLOGEPETRONA JULY 2009

Chemical Pre-treatment of Lignocellulosic Materials in Empty Fruit Bunch (EFB) From Palm Oil Solid Wastes for Bio-hydrogen Production

by

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CERTIFICATION OF APPROVAL

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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.

Mohd Saiful Abrar Bin Mohd Yusof

ABSTRACT

Palm oil solid waste consists of lignocellulosic materials which can be a useful product to produce bio-hydrogen. In this research, Empty Fruit Bunch (EFB) from palm oil solid wastes is used as a substrate. Pretreatment of lignocellulosic materials in EFB is one of the important processes in order to obtain high yield of bio-hydrogen conversion. Chemical pretreatment is applied here to remove lignin and break the cellulose and hemicelluloses into small compound so that the surface area of EFB can be increased. Four process variables that might affect the pretreatment performance which are acid concentration, particle size, temperature, stirring or agitation speed will be investigated. Diluted sulfuric acid treatment which will be used in this research is expected to hydrolyze EFB with result in high yield of monomer (glucose), change the structure of lignin and at the same time increase the surface area of biomass substrate for next process. The concentration of glucose in the pretreated sample can by determined by using UV/Vis Spectrophotometer. Thus, kinetic study for four effective parameters will be discussed in this report to have the understanding of reaction that occurred.

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TABLE OF CONTENT

CERTIFICATE	•	•	•	•	•	•	•	•	I
ABTRACT .	•	•	•	•	•	•	•	•	ш
ACKNOWLEDGE	MENT	•			•	•	•		IV
TABLE OF CONTI	ENT			•	•	•	•		V
LIST OF FIGURES			•	•	•			•	VIII
LIST OF TABLES									IX

CHAPTER 1:

INTRODUCTION	•	•	•	•	•	•	1

1.1	Background of Proj	ect	•	•	•	·	•	2
1.2	Problem Statement	•	•	•	•	•	•	4
1.3	Objectives .		•	•	•	•		4
1.4	Scope of Study							4

CHAPTER 2:

LITERATI	URE REVIEW/THEORY	•	•	•	6
2.1	Hydrogen Production from Biomass				6
2.2	Gasification			•	7

2.3	Effect of Feed Properties on Gasification	•	8
2.4	Pretreatment Method		8
2.5	Acid Pretreatment	•	10
2.6	Effective parameters in pretreatment of ligncellulosic	•	11
	2.6.1 Effect of Sulfuric Acid Concentration .		11
	2.6.2 Effect of Reaction Temperature		11
	2.6.3 Effect of Particle Size		12
	2.6.4 Effect of Stirring/ Agitation Speed	•	12
СНАРТЕН	R 3:		
METHODO	LOGY/PROJECT WORK	•	13
3.1	Overview of the project work		13
3.2	Preparation of Biomass Material (EFB)		14
3.3	Pretreatment Procedure	•	15
3.4	Preparation of DNS Reagent	•	16
3.5	Preparation of Glucose Standard Curve	•	17
СНАРТЕН	R 4:		
RESULT AN	ND DISCUSSION	•	18
4.1	Effect of sulfuric acid concentration		19

4.2	Effect of temperature	•	•	•	•	•	21
4.3	Effect of particle size.	•	u° ● ₂ =	•	•	•	26
	4.3.1 Shrinking core mod	el	•	•	•	•	27
	4.3.2 Diffusion through in	nert/ a	ash laye	er conti	rol .		29
4.4	Effect of agitation .	•	•	•	·	·	30
CHAPTER	15:						

RECOMMENDATION AND CONCLUSION				33
	•	•	-	

REFERENCE	·	•	•	•	•	•	•	•	34
APPENDICES									35

LIST OF FIGURES

Figure 1.1: Lignocellulosic material in Empty Fruit Bunch (EFB)	4 N	•	3
Figure 1.2: Cellulose Basic Structure [Antal, 1989]	•	•	3
Figure 1.4: Small Piece of Lignin Polymer [Antal, 1989] .			3
Figure 1.3: Hemicellulose Basic Structure [Antal, 1989] .	·		3
Figure 2.1: Gasification Process Diagram			7
Figure 2.2: Pretreatment of lignocelluloses		•	9
Figure 3.1: EFB was sieved 			14
Figure 3.2: EFB collected according to size	•	•	14
Figure 3.3: Extraction of oil from EFB using soxhlet .			14
Figure 3.4: 3, 5-dinitrosalicyclic acid reaction	•	•	16
Figure 3.5: Five sets of glucose standard solution from 1-5 mg/mL	2	•	17
Figure 3.6: Samples was boiled in a water bath for 5 minutes.			17
Figure 3.7: Samples then cooled for 10 minutes		•	17
Figure 3.8: Samples ready for analysis			17
Figure 3.9: Absorbance was measured in Spectrophotometer	•		17
Figure 4.1: Standard Curve of Glucose			18
Figure 4.2: Total reducing sugar with respect to acid concentration		•	20
using 250µm EFB, 150 rpm , 5% solid at 45°C			
Figure 4.3: Total reducing sugar with respect to temperature .			22

using 20% $H_2SO_4, 250 \mu m$ EFB, 150 rpm and 5% solid loading

Figure 4.4: Graph of ln k Vs 1/Temperature			•	24
Figure 4.5: Total reducing sugar with respect to particle	·		•	27
size using 20% $H_2SO_{4,}$ 5% solid loading and	150 rp	om at 45°	C	
Figure 4.6: Shrinking Core Model of Diffusion through ine	rt/ash	layer con	ntrol	29
Figure 4.7: Total reducing sugar with respect to agitation	•		•	31
using 20% H_2SO_4 , 250 μ m EFB and 5% solid 1	oading	5		

LIST OF TABLES

Table 4.1: Glucose concentration for 10% sulfuric acid in 60 minutes	19
Table 4.2: Glucose concentration for 20% sulfuric acid in 60 minutes	19
Table 4.3: Glucose concentration for 30% sulfuric acid in 60 minutes	20
Table 4.4: Glucose concentration for 35°C temperature in 60 minutes	21
Table 4.5: Glucose concentration for 45°C temperature in 60 minutes	21
Table 4.6: Glucose concentration for 55°C temperature in 60 minutes	22
Table 4.7: The effect of temperature to pretreatment methods on	25
Activation Energy and rate constant	
Table 4.8: Glucose concentration for particle size smaller than 500µm	26
Table 4.9: Glucose concentration for particle size smaller than 250 μ m	26
Table 4.10: Glucose concentration for particle size smaller than $45 \mu m$	27
Table 4.11: Glucose concentration for sample with no agitation	30
Table 4.12: Glucose concentration for sample with agitation speed of 150 rpm	31

CHAPTER 1

1.0 INTRODUCTION

Biomass can be defined as all the biological material produced and existing within an ecological system. Biomass as a renewable energy is defined as the organic matter contained in plant and animal based products which can be used as a source of stored chemical energy (Ralph, 2004). Biomass includes crop residues (e.g cereal straw, rice husks and bagasse), animal wastes, woodlot arising, forest residues, municipal solid waste and energy crops can be a main resource of biomass in the respective countries depending upon the available biomass resource.

Malaysia is one of the palm oil producers around the world besides Indonesia and some other countries in South East Asia. The annual rate of palm oil in Malaysia is estimated to be more than twelve million tones (Hussin *et al*, 2002). But the residue of the palm oil is not been completely utilized. It was reported that about 27% of total weight of fresh fruit bunch will be the crude oil and the portion left was solid wastes. Total biomass produced by palm oil industries is about 10% was counted as the crude palm oil while the rest was counted as the lignocellulosic wastes present as trunks, fronds, palm pressed fiber and empty fruit bunch (EFB) (Hussin *et al*, 2002). It has been believed that EFB consists of about 50% cellulose compared to other biomass resource such as rice husk, bagasse, rice straw, soybean straw and corn cobs.

Biomass can be converted to a synthesis gas by gasification. In its widest sense the term gasification covers the conversion of any carbonaceous fuel to a gaseous product with a usable heating value (Chris, 2003). Biomas syngas produced from gasification mainly consist of carbon monoxide, carbon dioxide and hydrogen. Hydrogen is the desired product which it has a lot of application in industries such as in power generation. However, conversion of hydrogen from biomass will not be high if the feedstock does not have certain characteristics which favor the gasification to produce more hydrogen.

1

1.1 Background of Project

Lignocellulosic materials predominantly contain a mixture of carbohydrate polymers which are cellulose, hemicelluloses and also lignin. The carbohydrate polymers are tightly bound to lignin mainly through hydrogen bonding and some covalent bonding. Cellulose is a linear polymer which the basic repeating unit is in fact cellobiose, a dimer of two-glucose unit. As glucose units are linked together into polymer chains, a molecule of water is lost, which makes the chemical formula $C_6H_{10}O_5$ for each monomer called glucan. The parallel polyglucan chains from numerous intraand intermolecular hydrogen bonds which result in a highly ordered crystalline structure of native cellulose, interspersed with less-ordered amorphous regions. Due to the crystallinity, the chemical attack on the cellulose is retarded. The cellulose typically accounts for some 40wt% of the lignocelluloses.

Hemicellulose consists of shorter polymers of various sugars that glue the cellulose bundles together. It is usually accounts for about 25wt% of lignocellulose. Unlike cellulose, hemicelluloses are not chemically homogeneous. It is relatively easily hydrolyzed by acids into their monomer components consisting of glucose, mannose, galactose, xylose, arabinose and small amount of rhamnose, glucoronic acid, methylglucoronic acid and galacturonic acid. Lignin is a very complex molecule which consist of a tri-dimensional polymer or propyl-phenol that embedded in and bound to the hemicelluloses. It provides rigidity to the structure and accounts about 20 wt% of lignocelluloses.

Lignins are extremely resistant to chemical and enzymatic degradation and it should be removed because it occludes polysaccharides. Fractionation of biomass into respective component of lignocellulosic material will help the gasification process in producing bio-hydrogen. It is believed that smaller particle of biomass will give better heat transfer through out the system. There are also other characteristics of biomass feedstock have to obtain in order to achieve high efficiency and selectivity of hydrogen

2

in gasification. This paper will discuss first step for getting the biomass ready to be used as feedstock for gasification process which is pretreatment.



Figure 1.1: Lignocellulosic material in Empty Fruit Bunch (EFB)











Figure 1.4: Small Piece of Lignin Polymer [Antal, 1989]

1.2 Problem Statement

In producing bio-hydrogen from biomass, it has to undergo gasification process which is favorable in producing hydrogen compared to other process that available. Biomass which is the feedstock of the process must meet certain criteria i.e small particle size, shape, etc. so that the gasification process will be efficient. This is where pretreatment of the biomass must be done before gasification process. But the challenge of using lingocellulosic waste as the biomass resource is it would not easy to break lignocellulosic into smaller compound (sugar) as to get larger surface area for gasification. Therefore, study has to be conducted to obtained pretreatment method with optimum conditions that surely will give high selectivity of hydrogen in gasification.

1.3 Objectives

The objectives of the research are to study the four effective parameters towards the hydrolysis of lignocellulosic material which are:

- 1. To study the effect of sulfuric acid concentration
- 2. To study the effect of reaction temperature
- 3. To study the effect of particle size
- 4. To study the effect of stirring/ agitation speed

1.4 Scope of Study

To achieve high yield of sugar which lead to high selectivity of hydrogen in downstream process, it required a pretreatment process. The goal of pretreatment is to decrease the crystallinity of cellulose structure and increase the surface area of EFB by breaking down the polymer structure of lignocellulosic material into its corresponding monomer. Basically, pretreatment method can be physical, chemical, biological and the combination of these three methods.

4

In this research, the focus is only on chemical pretreatment of lignocellulosic materials in EFB into its corresponding monomer. But somehow, the physical pretreatment must be applied before proceed to other process. Physical pretreatment includes ball milling, communition (mechanical reduction of biomass particulate size) and compression milling. Analysis of the result obtained will be carried out to determine the effectiveness of chemical used for the pretreatment process.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Hydrogen Production from Biomass

Approximately 95% of the hydrogen produced today comes from carbonaceous raw material, primarily fossil in origin. Dependence on fossil fuels as the main sources has led to serious energy crisis and environmental problems. The increasing demand of product from fossil resource will speed up the depletion of it and the combustion of fossil fuels can cause the global warming and acid rain. These two problems have influenced people to look for an alternative resource which are renewable and environmental friendly. Biomass is one of the most abundant renewable resources that have been used for centuries. Currently, biomass contributes about 12% of today world energy supply, while in many developing countries, it contributes 40-50% energy supply (Demirbas, 2001).

The majority of the existing plants were designed and constructed to produce a synthetic gas, consisting primarily of hydrogen and carbon monoxide (CO), which is used for the production of hydrogen or Fischer-Tropsche (F-T) syncrude. Hydrogen is then used to produce a wide variety of chemicals and fertilizers. The Fisher-Tropsch syncrude is used to manufacture transportation fuels, lube oils, and specialty waxes. Among the most recent plants are those designed to produce a synthetic gas suited for firing in gas turbines for the production of clean electric power production. Only a fraction of this hydrogen is currently used for energy purposes. The bulk serves as a chemical feedstock for petrochemical, food, electronics, and metallurgical processing industries.

2.2 Gasification

Gasification is a process for converting carbonaceous materials to a combustible or synthetic gas. In general, gasification involves the reaction of carbon with air, oxygen, steam, carbon dioxide, or a mixture of these gases at 1,300°F or higher to produce a gaseous product that can be used to provide electric power and heat or as a raw material for the synthesis of chemicals, liquid fuels, or other gaseous fuels such as hydrogen. In contrast to combustion processes, which work with excess air, gasification processes operate at substoichiometric conditions with the oxygen supply controlled (generally 35 percent of the amount of O_2 theoretically required for complete combustion or less) such that both heat and a new gaseous fuel are produced as the feed material is consumed.

Gasification is one of the thermochemical processes which is favorable for hydrogen production, feasible and receiving much attention in hydrogen production in recent years. The products of gasification will be H_2 , CH_4 , CO, CO_2 and other gases depending on the organic nature of the biomass for gasification. Liquid products include tar and oils also formed at room temperature and solid products which mainly composed of char plus other inert material. Methane and other hydrocarbon vapors produced can be steam reformed for more hydrogen production. In order to increase the hydrogen production, water-gas shift reaction can also be applied.



Figure 2.1: Gasification Process Diagram

2.3 Effect of Feed Properties on Gasification

Operating parameters of gasification process as well as the characteristic of the feedstock influence the composition of gasification product. Among the operating parameters, temperature and pressure of the gasifier have the greatest effect on the product composition. Physical properties of EFB, such as particle size and porosity might have significant effects on the kinetic of EFB gasification. As the particle size become smaller, specific contact area between the EFB and reaction gasses increases, resulting in faster reaction. Because the moisture content of green biomass can be quite high and can negatively impact the conversion of biomass to energy processes, predrying may be needed.

Moisture content of 10 to 20% is usually preferred. As the moisture content of biomass increases, the efficiency of thermal conversion process decreases. The shape of the feedstock which is usually come in pallet shape should be below than 20 mm for efficient gasification (Maa and Bailie, 1973). Besides that, the structure of the biomass feedstock influences the temperature distribution in gasification. High porosity of biomass gives large surface area for reaction and uniform temperature could be achieved. For all of characteristic discussed above, it is recommended that biomass has to be pretreated first before it can be gasified in the gasifier with high efficiency. Thus, pretreatment method will be studied in order to produce feedstock that has good effect on gasification process later on.

2.4 Pretreatment Method

The goal of pretreatment is to increase the surface area of biomass in order to have high efficiency of gasification process. Pretreatment fractionate lignocellulosic materials into its basic components. The large number of pretreatments used for lignocellulosic materials can be classified into groups as physical, physico-chemical, chemical and biological processes. Physical pretreatment such as chipping, grinding and milling only reduced the size of the biomass. However, it is important to undergo physical pretreatment first before proceed to other pretreatment methods. Physicochemical pretreatment consists of Steam Explosion (autohydrolysis) and Ammonia Fiber Explosion (AFEX) which in these two methods, biomass is treated in high pressure that can cause degradation of hemicellulose and lignin (Silverstein, 2004).

Chemical pretreatment generally can be acid treatment and alkaline treatment. Acid pretreatment can utilized either diluted or concentrated acids but each of them have advantages and disadvantage. Diluted acid is preferable because it is easy to handle and not extremely corrosive as compared to concentrated acid. This will be discussed further in next section. Alkaline solution is believed can removed lignin more efficient than acid solution. Biological pretreatment involves microorganism such as brown-, white- and soft-rot fungi that are used to degrade lignin and sollubilized hemicelluloses. However, it takes much longer time to degrade lignocellulosic material compared to acid and alkaline treatment.



Figure 2.2: Pretreatment of lignocellulose

2.5 Acid Pretreatment

Chemical pretreatment is referring to hydrolysis which basically an exposure of lignocellulosic materials in EFB to a chemical for a period of time and at a specific temperature which resulting monomer of cellulose and hemicellulose polymer. Acids are predominantly used in chemical pretreatment. Sulfuric acid is the most investigated acid besides hydrochloric acid. Basically, pretreatment by acid can be divided into two groups which are concentrated-acid hydrolysis and diluted-acid hydrolysis. Pretreatment using concentrated acid such as sulfuric acid and hydrochloric acid are reported to give higher conversion of sugar as compared to diluted acid (Ahindra, 2008). Furthermore, it does not need high temperature and pressure. But the drawbacks of the using concentrated acid are it is toxic, corrosive and hazardous which require reactors which are highly resistant to corrosion. It will result in high capital investment and maintenance cost in practical use. Furthermore, the environmental impact will give a limitation to apply this in industries.

Pretreatment with diluted-sulfuric acid is a favorable method because it gives high reaction rate and can recover up to 95% of lignocellulosic sugars but it depending upon the substrate used and condition applied (Ahindra, 2008). Sulfuric acid is typically used in 0.5%-1.0% concentration with variation in temperature and retention time. In diluted-acid hydrolysis, the hemicellulose fraction will depolymerized at temperature lower than cellulose fraction. If higher temperature or long retention times are applied, the monosaccharide formed will be further hydrolyzed to other compounds. Therefore, it is suggested to have two stages of process which called "pretreatment" for the first stage and "hydrolysis" for the second stage.

10

2.6 Effective parameters in pretreatment of ligncellulosic

2.6.1 Effect of Sulfuric Acid Concentration

It is reported that acid pretreatment can operate either under a high temperature and low acid concentration or under a low temperature and high acid concentration (Taherzadeh and Karimi, 2008). Concentrated-acid pretreatment need lower operating temperature compared to diluted-acid processes. However, drawback is that it is extremely corrosive and dangerous. The process will require special construction of reactor that can withstand the corrosion. Therefore, diluted-acid hydrolysis is probably the most commonly applied method among the chemical pretreatment methods. About 80% conversion of sugar can be obtained at 30% concentration of HCl in a reaction time of 60 minutes with 5% solid hydrolysis of EFB fiber (Najafpour *et al*, 2007). As the acid concentration increased, sugar concentration will increase as well due to more hydrogen ion formed in the solution which catalyzed the hydrolysis of cellulose.

2.6.2 Effect of Reaction Temperature

Temperature can gives significant effect on the conversion of sugar in hydrolysis. At an elevated temperature (e.g 140-190°C) and low concentration of acid (e.g 0.1-1% sulfuric acid), the diluted-acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis (Yang and Wyman, 2004). Almost 100% hemicelluloses removal is possible by diluted-acid pretreatment. The acid pretreatment for the batch system is not effective in dissolving lignin but it can disrupt lignin and increase the cellulose's susceptibility to enzymatic hydrolysis

2.6.3 Effect of Particle Size

It is found that the particle size of fibre greater than 0.4 mm gave the maximum concentration of xylose rather than a fibre with size smaller than 0.4 mm (Aziz *et al*, 2002). This was unexpected, as the smaller particles with larger surface areas should have produced more yields. The only possible explanation for this was the presence of impurities, such as debris and shell from grinding. As no xylose can produced from the shell, the higher the shell content, the lower would be the xylose concentration.

2.6.4 Effect of Stirring/ Agitation Speed

In chemical reaction, reactions are dependent on the reactants colliding in exactly the proper orientation and at sufficient force to allow the reaction to take place. The rate of the reaction for slow reaction can be increased by increasing the number of collisions in a given period of time and increasing the force of collisions by agitation with certain speed. Based on the experimental result, the agitation rate affects the mechanism of celluloytic attack which high agitation in the treatments, lasting 40 minutes or more, caused increasing cotton weight loss up to about 1.5% after 120 minutes (Paulo and Elmeida, 1996).

CHAPTER 3

3.0 METHODOLOGY/ PROJECT WORK

3.1 Overview of the project work

The experiment to study the effect four different process variables or parameters as stated above on total reducing sugar for EFB biomass has been referring to previous work made by researchers in biomass pretreatment especially research on the waste from palm oil. The conditions such as temperature, particle size and acid concentration was taken from the previous work done and it will be selected as standard condition in this experiment as it has been proved at previous work that these conditions gave the best result of yield conversion of glucose by using HCl. At the end of the experiment, it is expected to have better result as compared to the previous work done by the researcher respectively.

The pretreated sample is expected to contain glucose as the product from hydrolysis of lignocellulosic material in EFB by sulfuric acid. The sample was analyzed using UV/VIS spectrophotometer to determine the percent of absorbance which proportional to the concentration of glucose inside the pretreated sample. Calibration curve need to be prepared as the reference for the sample concentration. Dinitosacyclic acid (DNSA) reagent need to be added in the sample as the glucose will reduce 3, 5-dinitrosalicyclic acid (DNSA) to 3-amino-5-nitrosalicyclic acid. It uses the color conversion reaction from yellow to brown at wavelength 540 nm which glucose will absorbed the spectrum at this wavelength in UV/VIS Spectrophotometer. The conversion of moles DNS equals to moles of glucose.

3.2 Preparation of Biomass Material (EFB)

Palm Oil EFB obtains from FELCRA palm oil milling plant in Bidor, Perak. Initially, EFB was dried in an oven at 100°C for about 24 hours. Physical pretreatment was carried out using grinding machine in the laboratory in order to achieve a large surface area of cellulose for reaction with sulfuric acid. The solid collected was then sieved to get particle size which ranging from 500 μ m – 45 μ m. A preliminary analysis of sample was carried out before the pretreatment which measure the oil and moisture content of the EFB.

Soxhlet was used to extract some oil from EFB. The percent of oil content in EFB was determined by calculating the weight difference after the sample being put into soxhlet. Then, three grams of samples was placed in the convection oven overnight (or until constant weight was achieved) at 105°C and reweighed. The percent moisture was calculated with the following formula:

% Moisture = 100% -
$$\left(\frac{W_2 - W}{W_1 - W}\right) \times 100$$
 (3.1)

Where: W = weight of dish $W_1 = initial sample weight + dish weight (g)$ $W_2 = dried sample weight + dish weight (g)$



Figure 3.1: EFB was sieved



Figure 3.2: EFB collected according to size



Figure 3.3: Extraction of oil from EFB using soxhlet

3.3 Pretreatment Procedure

The experiment to study the effect of sulfuric acid concentration was carried out in a flask using 10%, 20% and 30% H₂SO₄ at 45°C. About 5% of EFB was charged into a 250 Erlenmeyer flask and placed in a temperature controlled water bath shaker with agitation speed of 150 rpm. The size of EFB particle used was 250 μ m. The reaction time was set for 1 hour. The hydrolyzate was collected and filtered through filter paper, Whatman #1. Then, the hydrolyzate will be 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 540 nm using 3, 5dinitrosalicycilc acid (DNSA) as a reducing chemical for glucose. The same procedure applied for others parameters.

The effects of pretreatment concentration, temperature, particle size, and agitation speed will be analyzed in term of reaction kinetic. The result will be discussed and justified. There will be four graphs presentation as to demonstrate the result obtained comprising:

- The total reducing sugar with respect to sulfuric acid concentration [10%, 20%, and 30% (w/v)] using 250 μm EFB, 5% solid loading, at 45°C with speed of 150 rpm
- II. The effect of temperature [35°C, 45°C and 55°C] on total reducing sugar using 250µm EFB at 20% NaOH and 5% solid with speed of 150 rpm
- III. The total reducing sugar with respect to particle size [500, 250, and 45 μ m] at 20% NaOH, 5% solid at 45°C with speed of 150 rpm
- IV. The effect of shaking/agitation speed [0 and 150 rpm] on total reducing sugar using 250µm at 20% NaOH, and 5% solid at 45°C

15

3.4 Preparation of DNS Reagent

The estimation of total reducing sugar (as glucose) is based on the Dinitrosalicyclic acid (DNS) method after miller (1959) and Ghose and Kostick (1970). A double beam UV/Vis spectrophotometer was used to measuring percent transmittance. Preparation of DNS reagent must be done before sample can be analyzed. 10.6 g Dintrosalicyclic acid will be added with 19.5g Sodium Hydroxide. The ingredients were dissolved in approximately 600-800 ml of distilled water and gently heated in a water bath at 80°C until a clear solution was obtained. Then, 306 g Roschell salt (sodium potassium tartarate), 7.5 ml Phenol (melted at 60°C) and 8.3g Sodium metabisulfate were then added. After dissolving these chemicals, the final volume was raised up to 1416 ml with distilled water. The reagent was then filtered through a large coarse glass filter and stored at room temperature in an amber color glass bottle to avoid photo oxidation. It is stable under conditions for about 6 months. However, all chemicals except 3, 5-dinitrosalicyclic acid were not available in the laboratory which make the DNS Reagent cannot be prepared. Samples were only be added with 3, 5-dinitrosalicyclic acid and observation was made.



Figure 3.4: 3, 5-dinitrosalicyclic acid reaction

3.5 Preparation of Glucose Standard Curve

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 and 2.0 ml of 0.001DNS reagent was added into each tube. The tube was placed in a boiling water bath for 5 minutes following Ghose (1969). Next, they were cooled at room temperature. A blank was run parallel replacing 2.0 ml of sample dilution with distilled water. The % transmittance was measured at 546 nm on a spectrophotometer. A graph was plotted taking the transmittance at the coordinate and sugar concentration.



Figure 3.5: Five sets of glucose standard solution from 1-5 mg/mL



Figure 3.6: Samples was boiled in a water bath for 5 minutes





Figure 3.9: Absorbance was measured in Spectrophotometer



Figure 3.8: Samples ready for analysis

Figure 3.7: Samples then cooled for 10 minutes

CHAPTER 4

4.0 RESULT AND DISCUSSION

Before EFB being pretreated by sulfuric acid, the EFB was subjected to undergo preliminary analysis on oil and water content. It was found that EFB contain about 13% of oil and 57% of water when it was freshly taken from the FELCRA palm oil milling. Four parameters should be studied such as acid concentration, reaction temperature, solid particle size and stirring effect. Each parameter will be evaluated based on production of sugar (glucose) at the end of the pretreatment. The sugar concentration in each sample will be determined by using UV-Vis Spectrophotometer

Hydrolysis of EFB was carried out in 10%, 20% and 30% of sulfuric acid at retention time of 60 minutes, 45°C temperature and 5% solid loading. To determine the concentration of sample, glucose standard curve need to be prepared first. The glucose standard curve obtained as shown below:



Figure 4.1: Standard Curve of Glucose

4.1 Effect of sulfuric acid concentration

For experiment to study the effect of sulfuric acid concentration, three sets of data which are 10%, 20% and 30% acid concentration obtained as shown below:

Result for 10% sulfuric acid

Table 4.1: Glucose concentration for 10% sulfuric acid in 60 minutes

Time, min	Concentration, mg/mL	Absorbance
0	0	0
10	0.425	0.516
20	0.777	0.633
30	0.847	0.656
40	1.653	0.924
50	1.786	0.968
60	1.853	0.99

Result for 20% sulfuric concentration

Table 4.2: Glucose concentration for 20% sulfuric acid in 60 minutes

Time, min	Concentration, mg/mL	Absorbance
0	0	0
10	1.312	0.811
20	1.823	0.98
30	1.936	1.018
40	2.395	1.17
50	2.464	1.193
60	2.666	1.26

Result for 30% sulfuric concentration

Time, min	Concentration, mg/mL	Absorbance
0	0	0
10	2.203	1.107
20	2.296	1.137
30	2.767	1.294
40	3.445	1.519
50	4.981	2.029
60	4.9	2.002

Table 4.3: Glucose concentration for 30% sulfuric acid in 60 minutes

Result from UV-Vis spectrophotometer showed that there was a significant difference in glucose production as the concentration of sulfuric acid increase from 10%-30%. These three experiment results can be represented in a graph as shown below:



Figure 4.2: Total reducing sugar with respect to acid concentration using 250µm EFB, 150 rpm , 5% solid at 45°C

From the graph obtained, sugar concentration was increased when concentration of acid increase. Higher concentration of acid will have more hydrogen ion which catalyzed the hydrolysis to be more rapid. Therefore, the breaking of glucosidic bounds will increase, causing the high conversion of hemicelluloses fraction into xylose. The cellulose fraction will be disrupted and glucose will be produced. In this study, only glucose concentration was investigated. At retention time of 60 minutes, high concentration of acid will speed up the reaction rate which results in high production of glucose.

4.2 Effect of temperature

For experiment to study the effect of temperature, three sets of data which are 35°C, 45°C and 55°C obtained as shown below:

Result for $T = 35^{\circ}C$

Time, min	Concentration, mg/mL	Absorbance
0	0	0
10	0.163	0.429
20	0.449	0.524
30	0.555	0.592
40	0.906	0.676
50	1.01	0.71
60	1.056	0.726

Table 4.4: Glucose concentration for 35°C temperature in 60 minutes

Result for $T = 45^{\circ}C$

Table 4.5: Glucose concentration for 45°C temperature in 60 minutes

Time, min	Concentration, mg/mL	Absorbance
0	0	0
10	0.322	0.482
20	0.9	0.674
30	1.61	0.91
40	2.68	1.265
50	2.904	1.339
60	3.031	1.361

<u>Result for $T = 55^{\circ}C$ </u>

Time, min	Concentration, mg/mL	Absorbance
0	0	0
10	0.865	0.662
20	1.761	0.945
30	2.962	1.359
40	4.639	1.916
50	4.833	1.98
60	4.943	2.017

Table 4.6: Glucose concentration for 55°C temperature in 60 minutes

These three experiment results can be represented in a graph as shown below:



Figure 4.3: Total reducing sugar with respect to temperature using 20% H₂SO₄, 250µm EFB, 150 rpm and 5% solid loading

The effect of temperature on acid hydrolysis is shown in Figure 7. A temperature of 35°C, 45°C and 55°C was studied. The sugar concentration at 35°C was 1.056 mg/mL. At 45°C, sugar concentration was about 3.031 mg/mL and the highest sugar concentration produced was at 55°C which about 4.943 mg/mL. From the result obtained, temperature did play an important role on rate of acid hydrolysis reaction which results in increasing of glucose production.

On the molecular level, the determining factor of the speed of a chemical reaction is the collisions between molecules. The more frequently they collide, the greater the likelihood that a reaction will take place. This seemingly involves bond breakage which must require energy. Thus, is not sufficient that the molecules just collide, but they must collide with sufficient energy such that the reaction may occur. This energy barrier that must be overcome is called the Activation Energy. By increasing the temperature one not only increases the number of collisions but one also increases the energy of the collisions and thus get a greater probability that some of those collisions have sufficient energy to overcome this barrier. There is another factor that comes into play called the orientation factor, though it is not very important in this reaction. This factor takes into account the number of collisions that occur at the correct location to effect breakage of the desired bond.

In 1889, Svante Arrhenius, demonstrated that the rate constant of a chemical reaction varies with temperature according to the equation

$$k = A e^{\frac{Ea}{RT}}$$

Where,

k = rate constant

A = Prexeponential term, a factor that includes collision frequency and orientation.

Ea = Activation energy.

R = Ideal Gas Constant (in J.mol⁻¹.K⁻¹)

T = Temperature (in K)

Take the natural log of the above equation:

 $\ln k = \ln A - (Ea/RT)$

Then, rewriting this slightly differently we get:

 $\ln k = (-Ea/R)(1/T) + \ln A$

Equation stated above is in fact the equation of a straight line that relates the natural logarithm of k to the inverse of the temperature. Thus if one were to plot $\ln k$ vs 1/T, one should obtain a straight line with a negative slope. The slope of this line being equal to -Ea/R. Thus, Ea can be calculated.



Figure 4.4: Graph of ln k Vs 1/Temperature

Graph of $\ln k$ vs $1/T_0$ was plotted in order to identify the activation energy E_a of the reaction. From the graph plotted, the activation energy was calculated from the slope obtained which is 7830. By substituting into Arrhenius equation, the activation energy calculated for this reaction is 65098.62 J/mol.

 Table 4.7: The effect of temperature to pretreatment methods on Activation Energy and rate constant

Sample	Temperature, ^o C	k_i , min- ¹	Ea, kJ/mole
1	35	0.0176	
2	45	0.0505	65.09
3	55	0.08238	

Table 4.7 present the rate constant temperature dependency and the role of acid concentration for 3 sets of experiments. According to Arrhenius law, the rate constant increased as the acid concentration and temperature increased. Besides, higher activation energy showed its kinetic behavior which high temperature produce high concentration of glucose.

4.3 Effect of particle size

Result for particle size smaller than 500 µm

Time, min	Concentration, mg/mL	Absorbance
0	0	0
10	0.163	0.429
20	1.047	0.723
30	1.183	0.763
40	1.439	0.853
50	1.694	0.937
60	2.111	1.076

Table 4.8: Glucose concentration for particle size smaller than 500µm

Result for particle size smaller than 250 µm

Table 4.9: Glucose concentration for particle size smaller than 250 µm

Time, min	Concentration,mg/ml	Absorbance
0	0	0
10	1.312	0.811
20	1.823	0.98
30	1.936	1.018
40	2.395	1.17
50	2.464	1.193
60	2.666	1.26

Result for particle size smaller than 45 µm

Time, min	Concentration, mg/mL	Absorbance
0	0	0
10	1.203	1.107
20	2.296	1.137
30	2.526	1.214
40	2.599	1.238
50	2.611	1.242
60	2.756	1.29

Table 4.10: Glucose concentration fo	r particle size smaller than 45µm
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4.3.1 Shrinking core model

The SCM was first developed by Yagi and Kunii, 1955. In the establishment of the SCM, the solid reactant is considered to be non-porous and is initially surrounded by a fluid film through which mass transfer occurs between the solid particle and the bulk of the fluid. As the reaction proceeds, an ash/inert layer forms around the unreacted core.

The general reaction shown in equation below is used in the development of the SCM. Detailed derivation of the shrinking core model can be found in Levenspiel book (1999).

Sulfuric acid (fluid) + EFB (solid) -----> glucose and solid products

The form of rate equation is determined by the control regime or the rate-limiting step which can be one of the following three:

1. Diffusion through the liquid film surrounding the particle. Let the time of a complete conversion of complete be τ . Thus, we obtained relationship of time with radius, R and conversion, X_B.

When the diffusion through liquid film controls,

$$\frac{t}{\tau} = X_B \qquad \text{Where } \tau = \frac{\rho_B R}{3bK_g C_{AB}}$$

2. Diffusion through ash/inert solid layer.

When the diffusion through inert/ash layer controls,

$$\frac{t}{\tau} = 1 - 3(1 - X_B)^{2/3} + 2(1 - X_B) \qquad \text{Where } \tau_{AD} = \frac{P_B R^2}{6b D_e C_{AB}}$$

3. Chemical reaction at the surface of the unreacted core.

When surface chemical reaction controls,

$$\frac{t}{\tau} = 1 - (1 - X_B)^{1/3} \qquad \text{Where } \tau_{rc} = \frac{\rho_B R}{b k C_{Ag}}$$

It is assumed that the solid particle is spherical and it reacts with the fluid isothermally and diffusion through as/inert solid layer to be the rate-controlling because lignin resist on chemical attack. Also, the concentration of the reacting fluid is assumed to be constant or in excess.

4.3.2 Diffusion through inert/ ash layer control



Figure 4.6: Shrinking Core Model of Diffusion through inert/ash layer control

In this experiment, diffusion of sulfuric acid into the cellulose so that hydrolysis occurred had to go through resistance of lignin and hemicelluloses which can be summarize in steps as shown below:

- Step 1: Diffusion of sulfuric acid through the film surrounding the particle to the surface of the solid.
- Step 2: Penetration and diffusion of sulfuric acid through the blanket of ash (lignin and hemicellulose) to the surface of the unreacted core (cellulose).
- Step 3: Reaction of sulfuric acid with cellulose at this reaction surface
- Step 4: Diffusion of glucose from cellulose through the ash back to exterior surface of the solid
- Step 5: Diffusion of glucose through the liquid film back into the sulfuric acid

When the diffusion through inert/ash layer controls,

$$\frac{t}{\tau} = 1 - 3(1 - X_B)^{2/3} + 2(1 - X_B)$$

 $\tau_{AD} = \frac{P_B R^2}{6b D_e C_{AB}}$ where R = initial particle radius

From the equation above, we obtain the relationship of time with radius and with conversion. As the initial particle radius decreases, the time required for particle to react completely will decrease. Thus, at retention time of 60 minutes, the smallest particle size will produce more glucose than the bigger particle.

4.4 Effect of agitation

Result of sample with no agitation

Time,min	Concentration, mg/mL	Absorbance
0	0	0
10	0.163	0.429
20	1.047	0.723
30	1.183	0.768
40	1.439	0.853
50	1.694	0.937
60	2.111	1.076

Table 4.11: Glucose concentration for sample with no agitation

Result of sample with agitation speed of 150 rpm

Time,min	Concentration,mg/ml	Absorbance
0	0	0
10	1.312	0.811
20	1.823	0.98
30	1.936	1.018
40	2.395	1.17
50	2.464	1.193
60	2.666	1.26







Reaction rates for process carried out in agitated vessels are frequently governed by interface mass transfer mechanism and rate controlling step is often mass transport across a liquid film. For sample with no agitation, it will be considered to have a case of liquid film-controlled mass transfer. For the sample with agitation, the diffusion of sulfuric acid through liquid film was fast. The various mass transfer mechanism which have been postulated to fit experimental data are based on Fick's first law, which applicable for steady state conditions, the following relationship is obtainable:

$$N = k (C_s - C)$$

The rate constant k in equation above can be interpreted as the ration of molecular diffusivity to a stagnant film thickness:

$$k = D/F$$

by this concept of film theory proposed by Lewis and Whitman in 1924, mass transfer into or out of a moving fluid phase occurs by molecular diffusion through a thin film of stagnant fluid at the phase boundary. Mass transfer through the stagnant film is presumed to be slower by orders of magnitude than in the bulk phase and controls the overall single phase rate. According to equation above, the rate of mass transfer is directly proportional to molecular diffusivity and diffusion is assume to occur only in a direction perpendicular to the interface. By having agitation in the process, particles will have more direct contact with sulfuric acid in perpendicular direction to the interface.

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATIONS

From the experiment results, it can be concluded that acid concentration has significant effect on production of glucose from hydrolysis of lignocellulosic material in EFB. As the concentration of acid increase, the glucose concentration will increase. The highest concentration glucose obtained was for 30% sulfuric acid (4.9 mg/mL glucose), followed by 20% sulfuric acid (2.666 mg/mL glucose) and 10% sulfuric acid (1.853 mg/mL glucose). Temperature also did play an important role in determination of the rate of reaction. The reaction rate constant was temperature dependent and value of activation energy can be obtained by using Arrhenius equation. The hydrolysis of EFB gave better conversion for particle smaller than 45µm as compared to 500µm. This was further discussed in kinetic study by using shrinking core model of non-catalytic fluid reaction. Mass transfer of liquid to solid phase is important in order to obtain fast reaction and to prevent diffusion through liquid film from become the controlling step. Thus, agitation is important to increase the mass transfer as well as increase the rate of reaction.

For future work, it is recommended that the optimum condition of hydrolysis of should be obtained so that pretreatment can be done in effective and economical way. The Total Dissolve Solid (TDS) for each sample should be investigated so that glucose can be identified in term of weight percentage from hydrolyzate. Method of glucose determination using UV-Vis Spectrophotometer should improve so that calorimetric method can give more accurate result.

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APPENDICES

APPENDIX A: Spectrophotometric Analysis on Concentration of Glucose

Standard Curve of Glucose



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Sample Reading for Temperature Effect (35°C, 45°C & 55°C)

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Sample Reading for Particle Size (500µm & 45 µm)





Sample Reading for non-agitation

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Std 2	Standard		4.0000	1.7043	1.00	4	4 0000	A					1
Std 3	Standard		3.0000	1.4044	1.00		1000						1
Std 4	Standard		2.0000	1.1134	1.00 🗸		0.4947				I		
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mple Table - (A	ctive)						1.141		Samp	le Graph			
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sample1	Unknown		0.163	0.429	a.	.54							1
sample2	Unknown		1.047	0.723			1.000						1
sample3	Unknown		1.183	0.768								•	
sample4	Unknown		1.439	0.853									
sample5	Unknown		1.694	0.937			0.800		1.1				+
sample6	Unknown		2.111	1.076		1							
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APPENDIX B: Material and Equipments

Water Bath Shaker model Innova 3100, made in New Jersey USA by New



Brunswick Scientific

UV/ Vis SpectrophotometerUV-VIS Spectrophotometer with model number UV3150, made in Japan by Shimadzu Corporation



Empty Fruit Bunch



EFB in Erlenmayer Flask

