BIODELINIFICATION AS PRETREATMENT TO ACID HYDROLYSIS OF OIL PALM TRUNK (OPT): KINETIC STUDY

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SUPERVISOR'S DECLARATION

"I hereby declare that I have checked this project and in my opinion, this project is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering and Natural Resources."

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Date: 18 FEBRUARY 2012

STUDENT'S DECLARATION

I declare that this thesis entitled "Biodelignification as Pretreatment to Acid Hydrolysis of Oil Pam Trunk: Kinetic Study" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidate of any other degree.

Signature :

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Date : 2013

DEDICATION

Special dedication to,

My parents

Wan Yusoff bin Wan Kadir and Hanim bt Khalid

My beloved brothers and sisters

Wan Noor Ain, Wan Nur Aqilah and Wan Mohd Asyraf

My friends

Aiza bt Abd Rahman and all of my friends

My co-supervisor

Zulsyazwan bin Ahmad Khushairi

My supervisor

Dr Norazwina bt Zainol

For her kindness in helping me all the way

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ABSTRACT

Acid hydrolysis is important to breakdown cellulose into its constituent sugar such as glucose. Biodelignification is one example of pretreatment. Pretreatment is applied to make the cellulose accessible to hydrolysis for further conversion such as sugar. Kinetic study is important to determine kinetic parameter for production of glucose by acid hydrolysis. The main purpose of this research is to study kinetic acid hydrolysis of OPT to produce glucose. The methodology of this research can be summarized as follow. Firstly, for untreated OPT, the research is started by run acid hydrolysis to OPT. Two stage of acid hydrolysis is applied. In this method, first, OPT is submerged in 60% sulfuric acid for 30 min and respectively at 60°C. Next, OPT is applied with 30% acid hydrolysis for 60 min and respectively at 80°C. For treated OPT, biodelignification is applied to OPT and continued with acid hydrolysis as mentioned in untreated OPT. Optimum conditions for biodelignification is as follow: temperature at 25.16°C, 7.54 pH values, 2.38 mL/12 hour moisture content and 1:2 fungi to medium ratio. RK fourth order is used to solve ordinary differential equation and the kinetic parameter obtained is analyzed. K_1, K_2 and Y_{max} are the kinetic parameters in this study which means K_1 is biomass decomposition rate, K_2 is sugar release rate and Y_{max} is glucose production. After analyze data, kinetic parameters value for treated OPT are Y_{max} = 5.266 g/l, K_1 = 0.011454 min^{-1} and K_2 = 0.015036 min^{-1} whereas for untreated OPT is Y_{max} = 4.878 g/l, K_1 = 0.007774 min^{-1} and K_2= 0.012584 min^{-1}. For conclusion, results showed that acid hydrolysis with biodelignification pretreatment give better result of kinetic parameter value this is because treated OPT have higher value of K_1, K_2 and Y_{max}. High value of K_1 means high rate to degrade biomass to produce glucose whereas high value of K_2 means that the faster time needed to achieve glucose production and high value of Y_{max} shows high glucose production. For future study, quantity of glucose obtained can be maximized by using this kinetic parameters obtained.

ABSTRAK

Hidrolisis asid adalah proses yang penting untuk memecahkan selulosa kepada konstituen gula seperti glukosa. Biodelignifikasi adalah salah satu contoh prarawatan. Prarawatan digunakan untuk membolehkan selulosa diakses oleh proses hidrolisis. Kajian kinetik adalah penting untuk menentukan parameter kinetik. Tujuan utama kajian ini adalah untuk mengkaji hidrolisis asid kinetik OPT untuk menghasilkan glukosa. Metodologi kajian ini boleh dirumuskan seperti berikut, terdapat OPT yang dirawat dan OPT yang tidak dirawat. Untuk OPT yang tidak dirawat, hanya diaplikasi dengan proses hidrolisis asid. Didalam proses hidrolisis asid terdapat dua langkah. Pertama, OPT akan direndam dalam 60% asid sulfurik selama 30minit dan pada suhu 60°C. Kedua, OPT akan direndam dalam 30% hidrolisis asid selama 60minit dan pada suhu 80°C. Untuk OPT yang dirawat, OPT diaplikasi dengan proses biodelignifikasi dan seterusnya diteruskan dengan proses hidrolisis asid. Keadaan optimum untuk biodelignifikasi adalah seperti berikut: 25.16°C, pH 7.54, kandungan lembapan 2.38 mL/12 jam dan nisbah kulat per media adalah 1:2. RK peringkat keempat digunakan untuk menganalisa persamaan kajian kinetik. Parameter kinetik adalah seperti berikut: K_1, K_2 dan Y_{max}. K_1 adalah kadar penguraian biojisim, K_2 pula adalah kadar pembebasan gula dan seterusnya Y_{max} adalah pengeluaran glukosa yang maksima. Selepas data dianalisa, nilai parameter kinetik yang diperolehi untuk OPT dirawat adalah Y_{max} = 5.266 g/l, K_1 = 0.011454min^{-1} dan K_2 = 0.015036min^{-1} manakala untuk OPT tanpa rawatan adalah seperti berikut Y_{max} = 4.878 g/l, K_1= 0.007774min^{-1} dan K_2 = 0.012584min^{-1}. Kesimpulannya, hidrolisis asid dengan prarawatan biodelignifikasi memberi hasil yang lebih baik kerana parameter kinetik menunjukkan nilai yang tinggi. Nilai K_1 yang tinggi bermaksud kadar yang tinggi untuk merendahkan biojisim untuk penghasilan glukosa manakala nilai K_2 yang tinggi bermakna masa yang lebih cepat diperlukan untuk pengeluaran glukosa dan nilai Y_{max} yang tinggi menunjukkan pengeluaran glukosa yang maksima.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

%	Percentage
ml	Milliliter
g	Gram
°C	Degree Celsius
g/l	Gram per litre
min^{-1}	Per minute
min	Minute
h	Hours
rpm	Revolutions per minute
mmol/L	Millimole per litre
w/w	Weight over weight

LIST OF ABBREVIATIONS

OPT	Oil palm trunk
P. ostreatus	*Pleurotus ostreatus*
RK	Runge kutta
H_2SO_4	Sulfuric acid
HCL	Hydrochloric acid
EFB	Empty fruit bunch
O_2	Oxygen
CO_2	Carbon dioxide
NaOH	Sodium hydroxide
H_2O_2	Hydrogen peroxide
APEX	Ammonia fiber explosion
K_1	Biomass decomposition rate
K_2	Sugar release rate
Y_{max}	Highest glucose production

LIST OF APPENDICES

APPENDICES A

Materials

1. Oil palm trunk
2. Sulfuric acid
3. NaOH
4. Dinitrosalicyclic (DNS) reagent
5. Kalium tartarate
6. Oyster mushroom

Apparatus

1. Uv-vis spectrophotometer
2. Water bath
3. pH meter

Methodology

1) Standard solution of glucose is prepared
2) OPT is cut into small pieces and dried in the autoclave incubator
3) nine conical flask is prepared and added 5g OPT each
4) For the first 30 minutes, added 22.64 ml 60% sulfuric acid in each for 30 min and respectively at 60°C.
5) Next, added 22.64 ml of water to each sample for the 30% acid concentration steps for 60 min and respectively at 80°C.
6) The sample is filter to obtain the filtrate and the residue is weighted.
7) The filtrate is neutralized with NaOH and DNS method is applied.
8) All samples are analyzed using uv-vis spectrophotometer.
9) The result obtained is recorded and analyze for kinetic study using excel solver.

Standard Curve of Glucose

According to Wang (n.d) dinitrosalicylic colorimetric method (DNS) method, tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the aldehyde functional group present in, for example, glucose and the ketone functional group in fructose. The absorbance is measured with a spectrophotometer at 575 nm. In the DNS method, it is important to obtain a standard curve first. Standard curves represent the relationship between two quantities. In this experiment, by referring to standard curve, the glucose concentration of sample can be determined.

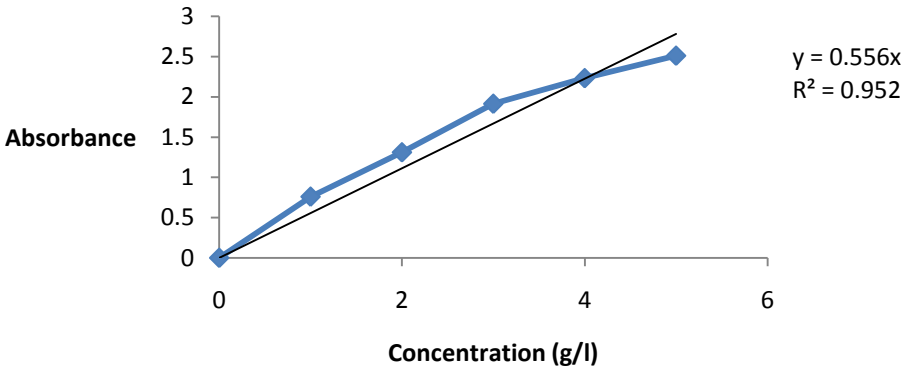

Figure A1 Standard curve of glucose

From figure A1, an equation of linear regression is obtained which is:-

$$y = 0.556x \qquad \text{(A1)}$$

Where: y = absorbance

x = glucose concentration

By referring to equation (A1), the glucose concentration of unknown sample is obtained.

Table A1 Result of untreated OPT

Time (min)	Glucose concentration (g/l)	Mass (g)
0	0	5
10	2.317	3.312
20	3.397	2.397
30	4.195	1.673
40	4.288	1.009
50	4.596	0.906
60	4.578	0.671
70	4.878	0.45
80	4.842	0.305
90	4.841	0.180

In this experiment the manipulated data is time with the interval 10 minutes each. Glucose concentration and dry weight data must be recorded to solve for kinetic model. Based on Table a.1, the maximum glucose concentration of untreated OPT is 4.878 g/l whereas the minimum dry weight of OPT is 0.180g.

Table A2 Result of treated OPT

Time (min)	Glucose concentration (g/l)	Mass (g)
0	0	5
10	2.932	2.587
20	4.284	1.621
30	4.858	1.003
40	5.067	0.4334
50	5.183	0.3745
60	5.212	0.1782
70	5.266	0.1028
80	5.167	0.09231
90	5.015	0.06112

From Table A2, the maximum glucose concentration of treated OPT is 5.266 g/l whereas the minimum dry weight OPT obtained is 0.06112g.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Malaysia is one of the world's top producers of oil palm product. Oil palm tree start bearing fruits after 30 months of field planting and will continue to be productive for the next 20 to 30 years, thus ensuring a consistent supply of oil. Around 30 years after planting, palm tree will be cut and palm tree trunk is one of the components of palm tree that will dispose. Thus, amount of this lignocellulosic waste has increased proportionally with increase of oil palm tree planting. To reduce the waste of this lignocellulosic biomass toward environment come the initiative to recycle this lignocellulosic waste to give a lot of advantages if this lignocellulosic waste is process. Oil palm trunk is one of hardwood that rich with cellulose and monomer sugar such as glucose. Thus, oil

palm trunk is called as lignocellulosic biomass because rich with cellulose.

Lignocellulosic waste such as oil palm trunk waste contains cellulose, hemicellulose and lignin. To extract sugar from oil palm trunk (OPT) a process called acid hydrolysis is apply. This acid hydrolysis process can be observed by using kinetic study. Ordinary differential equation and excel solver is used to solve the kinetic equation. For better result, it is believe that the oil palm trunk must be treat with biodelignification method as pretreatment to acid hydrolysis to remove lignin content. Thus, acid hydrolysis can run the process of release glucose from OPT efficiently.

1.2 Problem Statements

Malaysia produce large amount of palm oil and wooden furniture. Thus, amount of lignocellulosic material waste is also high. By using OPT as raw material to extract glucose, environmental problem of increasingly amount of this lignocellulosic biomass can be reduced. Besides that, acid hydrolysis is needed to extract glucose from OPT but the acid hydrolysis process is believed focus much on degradation of lignin in OPT. Hence, amount of glucose that can be extracting is also less. To solve this problem, lignin content in the oil palm trunk must be

degrade first, thus amount of lignin in the oil trunk will be less and consequently, acid hydrolysis can focus on extraction of glucose from OPT. The comparison of glucose content between treated OPT with biodelignification and untreated OPT can be done using kinetic study. Kinetic equation of acid hydrolysis of hardwood will be applied. Thus, comparison amount of glucose content can be observed.

1.3 Research Objective

The main purpose of this research is

i. To study kinetic acid hydrolysis of OPT to produce glucose.

1.4 Research Scopes

The scopes of this study are:

i. To apply a type of local white rod fungi (*pleurotus ostreatus*) called oyster mushroom for biodelignification process as pretreatment to acid hydrolysis.

ii. To compare the kinetic parameter of acid hydrolysis with pretreatment and without pretreatment by using kinetic study

iii. Kinetic study can be done by solve the equation chosen using RK fourth order and compare the value of kinetic parameters from acid hydrolysis of untreated OPT and treated OPT.

1.5 Rationale and Significant

By using this study, biodelignification as pretreatment to acid hydrolysis of OPT can be observe whether efficient enough to be apply. The efficiency of acid hydrolysis of untreated OPT and treated OPT could be determine from this kinetic study. This can be achieved by compare the kinetic parameters of treated and treated OPT which are K_1, K_2 and Y_{max}. Higher value of these three kinetic parameters are important as high value of K_1 means high rate to degrade biomass to produce glucose whereas K_2 shows that the faster time needed to achieve glucose production. Despite of that, higher value of Y_{max} is importance as high glucose production is needed for further application. Hence, for future study, quantity of glucose obtained can be maximized when kinetic parameters is successfully obtained.

CHAPTER 2

LITERATURE REVIEW

2.1 Acid Hydrolysis

Common acid used to treat lignocelluloses material are H_2SO_4 and HCL. Cellulose is the most abundant organic molecule however its susceptibility to hydrolysis is restricted due to the rigid lignin and hemicellulose protection. Hemicellulose can be readily hydrolyzed by dilute acids under moderate conditions, but much more extreme conditions are needed for cellulose hydrolysis. A pretreatment process is needed to improve its accessibility to hydrolytic enzymes (Mosier et al, 2005). The goal of pretreatment is to make the cellulose accessible to hydrolysis for further conversion such as its constituent sugars (Parveen et al, 2009). The factors affecting the hydrolysis of cellulose include porosity (accessible surface area) of the biomass materials, cellulose fiber crystalline, and content of both lignin and hemicellulose. The presence of

lignin and hemicellulose makes the accessibility of cellulose enzymes and acids to cellulose more difficult, thus reducing the efficiency of the hydrolysis process. Pretreatment is required to alter the size and structure of the biomass, as well as its chemical composition, so that the hydrolysis of the carbohydrate fraction to monomer sugars can be achieved rapidly and with greater yields.

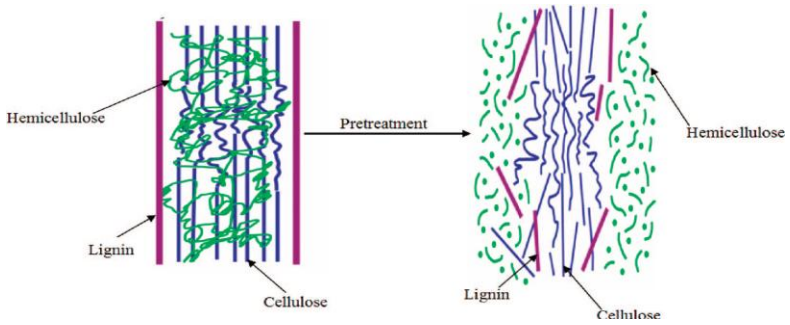

Figure 2.1 Schematic of the role of pretreatment

Ghasem et al (2007), studied single stage of acid hydrolysis process of palm oil empty fruit bunch (EFB) for production of fermentable sugar. This research was carried out under moderate temperature which is 45°C and at ambient pressure. The uses of high acid concentration for acid hydrolysis improved the reaction rate and sugar yield. Therefore, the sugar yield was found to be dependent on acid concentration and the employed temperature as well. The result shows that, for a reaction time of 40 minutes, 5 % EFB solid with 15, 20, 25 and

30 percent of HCl, EFB lignocelluloses fibers conversion of 36, 60, 65 and 80 % were achieved, respectively.

Azmalisa et al (2010), studied enzymatic hydrolysis process of the oil palm trunk fibers which can be converted into reducing sugars and subsequently be fermented to ethanol by suitable microorganisms. In this study, the conversion of cellulose to glucose with the help of cellulose enzyme accellerase TM 1000 was carried out. The results from Azmalisa et al study show that optimum conditions derived via RSM were: reaction time 13.5 h, temperature 40.8 °C, agitation rate of 167 rpm and amount of enzyme 0.4 ml. The experimental yield of glucose found to be 12.60 mmol/L under optimum condition, which compared well to the maximum predicted value, 15.15 mmol/L based on 0.5 g of substrate.

Ng et al (2011) aimed to determine total extractable starch and sugar content from OPT by using steeping method and dilute acid hydrolysis. Xylose yield the highest production using steeping method while for sugar yield, hydrolysis efficiency of 82% was obtained for conversion of OPT to glucose using two stage concentrated sulfuric acid hydrolysis. Ng et al 2011 have come to conclusion that OPT can be considered as resource of substantial amount of starch and sugar.

Anil et al (2011), studied on dilute acid pretreatment of oil seed rape straw for bioethanol production. The objective was to investigate the effect of biomass loading, acid concentration and pretreatment time on yield of sugar obtained after acid hydrolysis. The result of highest concentration of glucose is at 90 min pretreated time with glucan conversion efficiency of 81% whereas for highest concentration of sugar concentration was achieved at pretreatment of 60 min.

Chin et al (2011), studied production of glucose from oil palm trunk and sawdust of rubber wood and mixed hardwood by acid hydrolysis. This research served to identify the optimum two stage concentrated acid hydrolysis condition that can convert these three lignocellulosic biomass to glucose efficiently. Two stages concentrated sulfuric acid hydrolysis process using different acid concentration and reaction time were performed on those lignocellulosic biomass samples. The optimum results for oil palm trunk, rubber wood and mixed hardwood sawdust were obtained by using 60% acid concentration reacted for 30 min during first stage hydrolysis and subsequently followed by another 60 min reaction time with 30% acid concentration during the second stage hydrolysis. The results showed that oil palm trunk has a higher glucose conversion yield than those of rubber wood sawdust and mixed hardwood sawdust.

2.2 Raw Materials

2.2.1 Sago

Tapioca Sago is generally known as sago. Its Botanical name is "Manihot Esculenta Crantz Syn. Utilissima". This is a well known crop that is recognized by several names in the various regions where it is consumed. It is known as tapioca in India and Malaysia (FAO, 1998). Tapioca Root is the basic raw material for Sago and starch. There are about 30% to 35% starch contents generally in Indian tapioca root. Tapioca root has a high resistance to plant disease and high tolerance to extreme stress conditions such as periods of drought and poor soils. Fresh roots contain about 60 - 70% moisture, 7 - 12% protein, 5 - 13% starch (32 - 35% total carbohydrate) and trace amounts of fat (Lancaster et al., 1982; Jackson, 1990; FAO, 1998). The high starch and moisture content render it extremely perishable (Hahn 1989; Mlingi et al., 1996). Eventually, sago plant cannot be raw material in this study because will compete with food supply.

2.2.2 Rubber wood

Malaysian rubber industry has evolved through the years and transformed itself into a more integrated industry. About 80% of total wood furniture exported from Malaysia comes from rubber wood. Wood output can be obtained from planting rubber forest plantation based on 15 years cycle. Wood from tree has been traditionally regarded as waste. Total export value of rubber wood product has given by 39.44% in 2009 compared to 2000. Rubber wood is referred as an environmental friendly material with low price but its sustainable supply becoming a major concern nowadays (Ratnasingam, 2011). Despite of that, the glucose yield is lower that OPT (Chin, 2011).

2.2.3 Palm Tree

According to Alfreed (2007), palm tree is endogenous or in growing plants belonging to the same great division of vegetable kingdom as grass, bamboos, lilies and pineapple. Palm tree are almost exclusively tropical plants and very few species being found in temperate zone. The trunks of some are almost perfectly smooth, others rough with concentric rings or clothed with a woven or hairy fibrous covering, which bind together the sheathing basses of the fallen leaves.

2.2.4 Oil Palm Trunk (OPT)

OPT is a part of palm tree which will be used in this study. In this study, OPT is categorized under hardwood group. Hardwood is wood from angiosperm trees which means ovules are enclosed in an ovary and develops into the fruits after fertilizations. Hardwood is also dicotyledonous tree, compact wood, and has a more complex structure than softwood. OPT is an example of lignocellulosic material. Approximately 90% of the dry weight of most plant materials is stored in the form of cellulose, hemicellulose, lignin, and pectin (Parveen et al, 2009). The amount of waste for oil palm tree (*Elaeis guineensis*) is estimated to be around 33 million tones including empty fruit bunches, fibers and shells (IMPOB, 2009b; Mohamed and Lee, 2006). Besides that, in 2007, as much as 10, 827 tones of OPT are obtained as waste showing that these OPT are the largest contributors in waste from the agricultural industry (Goh et al., 2009). A study done by Run Chang and Tomkinson (2001) stated that the chemical composition (% dry weight, w/w) of oil palm trunk fiber is the following: cellulose 41.2%, hemicellulose 34.4%, lignin 17.1%, ash 3.4%, extractives 0.5%, and ethanol soluble 2.3%.

Table 2.1 Chemical Composition of Different Parts of the Oil Palm

Parts of oil palm	Extractives	Chemical Composition (%)		
		Holocellulose	Alpha Cellulose	Lignin
Bark	10.00	77.82	18.87	21.85
Leaves	20.60	47.7	44.53	27.35
Frond	3.50	83.13	47.76	20.15
Mid-part of trunk	14.50	72.6	50.21	20.15
Core-part of trunk	9.10	50.73	43.06	22.75
Frond	1.40	82.2	47.60	15.20
Trunk	5.35	73.06	41.02	24.51
Hardwood	0.1-7.7	71-89	31-64	14-34
Softwood	0.2-8.5	60-80	30-60	21-37

Source: Rokiah et al. (2011)

2.3 Delignification Processes as Pretreatment to Acid Hydrolysis

There are several methods delignification of hardwood but every method has their own characteristics which can be classify to advantage and disadvantage. Delignification is a process to remove the lignin from the cellulose and hemicelluloses in the lignocellulosic material. Delignification can be roughly divided into different categories: physical

(milling and grinding), physicochemical (steam pretreatment/auto hydrolysis, hydrothermolysis, and wet oxidation), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), biological, electrical, or a combination of these.

2.3.1 Physical Delignification

First delignification introduce is physical delignification which also call as mechanical comminution. Physical delignification is combination of chipping, grinding and milling applied to reduce cellulose crystallinity. This method is far too expensive to be used in a full scale process. Other method is Pyrolysis. In pyrolysis, cellulose rapidly decomposes to gaseous product and residual char when biomass is treated at temperature higher than 300°C. The process is enhanced when carried out in a presence of $O_{2.}$ Physicochemical delignification which called steam explosion is the most commonly used for pretreatment of lignocellulosic material. Biomass is treated with high pressure saturated steam and then pressure is suddenly reduced, which makes the materials undergo explosive decomposition. Addition of sulfuric acid can improve the hydrolysis. This is one of the most cost effective delignification for hardwood.

2.3.2 Chemical Delignification

Example of chemical pretreatment is Ammonia fiber explosion (AFEX) method. This process is by exposed lignocellulosic material to liquid ammonia at high temperature and pressure for some period of time and pressure suddenly reduces. This method is very similar to steam explosion, the difference just addition of ammonia (Kumar et al, 2009). AFEX is very effective for biomass with higher lignin content such as wood. Besides that, is carbon dioxide explosion by using supercritical CO_2 explosion. The temperature used is lower than steam explosion. Hypothetically, CO_2 forms carbonic acid when dissolved in water and acid can increase hydrolysis rate (Kumar et al, 2009). Since this method operated at low temperature, it does not degrade sugar. This method is more costly than APEX. Another method to be discuses is ozonolysis method. In this method, hemicellulose is slightly affected but cellulose not. This method is carried out at room temperature and normal pressure. The important thing is this method can be designed to minimize the environmental pollution. Large amount of ozone is required thus this is expensive process (Tabil et al, 2011).

2.3.3 Biodelignification

Biodelignification is an example of pretreatment of acid hydrolysis. Microorganism is use to treat lignin and in the same time enhance enzymatic hydrolysis. According to Bak (2010) and Taniguchi (2005) this fungi produce enzyme that help to degrade lignin and access cell wall of carbohydrates. This method is done by allowing the fungus to feed on the biomass for a period of several weeks. Later, the biomass is either subjected to a second mild form of pretreatment or is sent directly to hydrolysis (Keller, 2003). The advantages of biodelignification that attract researcher are these processes provide environmental friendly and energy sufficient. The advantages of biodelignification are simple techniques, low energy requirement, no or reduced output of waste stream and reduced downstream processing costs (Keller et al, 2003; Nigam and Pandey, 2009)

2.4 Methods of Biodelignification

There are several microorganisms that can be use including white, brown, soft rod fungi and some ruminant bacteria (Caixia and Yebo, 2012). Brown rot will attack cellulose mainly whereas white and soft rod fungi attack both cellulose and lignin (Parveen et al, 2009). The white-rot fungus has been selected because this fungus is able to produce laccase

and manganese peroxidase (MnP) isoenzymes, hemicellulases and a poor complex of cellulases. These enzymes are regulated by carbon and nitrogen sources (Parveen et al, 2009). Other than that, *Pleurotus Ostreatus* has investigated for their ability to produce various lignolytic and cellulolytic enzymes such as laccase, lignin peroxidase, xylanase, endo-1, and 4-β-D-glucanase (CMCase) (Reddy et al, 2003).

2.4.1 Soft-rot Fungi

In soft-rot fungi decay, mainly cellulose and hemicellulose are degraded, while lignin degradation is restricted. Process is typically occurring in wood of high water content and high nitrogen content. Soft rot fungi look like brown rot. They are most commonly found in rotting window frames, wet floor boards and fence posts where nitrogen is recruited from soil or from atmospheric contamination. Some of these fungi are common decomposers of cellulose in soil and they are the least specialized of the wood decaying fungi. Soft-rot is characteristic to wood in wet environments such as railway slippers, poles, piles, ship and boat wood. In buildings, soft-rot is found in moist window frames. Both softwoods and hardwoods are attacked by soft-rot fungi. Characteristic species are *Chaetomium Globosum*, *Richurus Spiralis* and *Phialophora Mutabilis* (Schultz et al, 2003).

2.4.2 Brown Rot Fungi

Brown rot is a type of wood decay caused exclusively by members of basidiomycotine. An example of brown ort fungi is *Laetiporeus sulfurous.* Cellulose and hemicellulose are broken down in the wood substrate, while lignin remains preserved in a slightly modified form (Rayber & Boddy, 1998). Cellulose is broken down by hydrogen peroxide (H_2O_2) that is produced during the break-down of hemicellulose. Wood affected by brown rot fungi is usually dry, fragile and readily crumbles into cubes. Brown rot is also generally more serious than white rot. In contrast to white rot fungi, most brown rot fungi lack extra cellulose phenoloxidases.

2.4.3 White Rot Fungi

Lignin degradation by white-rot fungi has received considerable attention as a means for reducing accumulation of lignocellulosic wastes in the environment. Wood affected by white rot normally does not crack across the grain and will only shrink and collapse when severely degraded. Example of white rot fungi are *phanerochaete chrysosporium* and *coriolus versicolor*. White rot fungi produce various isoforms of extracellular oxidases including laccase, manganase peroxidase (MnP) and lignin peroxidases (LiP). These enzymes are involved in the

degradation of lignin in natural lignocellulosic substrate (Dirk et al, 2003). Some white rot fungi can produce all this three enzymes while others produce only one or two of them (Hataka et al, 1994). The most common lignolytic peroxidase produces by almost white rot fungi basidiomycetes is manganese peroxidases (MnP). Lignin peroxidases (LiP) catalyze the oxidation of non phenolic aromatic lignin and similar compounds. LiP is well known as part of ligninolytic system both of aphyllophoralic and agaricalic fungi (Glenn & Gold, 1983). The role of LiP could be the further transformation of lignin fragments which are initially released by MnP. Another enzyme is laccase. Laccase catalyze the oxidation of variety of aromatic hydrogen donors with the concomitant reduction of O_2 to water. Laccase also can decarbocylate and attack methoxyl group. (Dirk et al, 2003). Further studies on white rot fungi ability to degrade lignin are tested and it proved the most effective basidiomycetes for biological pretreatment of lignocellulosic material (Fan et al, 1987).

2.4.4 *Pleurotus Ostreatus* (Oyster Mushroom)

Oyster mushroom belongs to class basidiomycetes, subclass hollobasidiomycetidae, and order agricals. The Latin *Pleurotus* means “beside the ear”, and *ostreatus* means “oystershaped” whereas the common name “oyster mushroom” stems from the white, shell like

appearance of the fruiting body. Moreover, oyster mushroom is an edible mushroom with excellent flavor, taste and is considered to be rich in protein, fiber, carbohydrates, vitamins and minerals. Moreover, it can be grown on agricultural and industrial waste (Shah et al, 2004). This mushroom represents a valuable source of protein for rural populations and its production has increased significantly in recent years (Chang, 1999). *Pleutotus ostreatus* is primary decomposers of hardwoods in North America. One of the most important aspects of *Pleurotus* spp is related to the use of their ligninolytic system for a variety of applications, such as the bioconversion of agricultural wastes into valuable products for animal feed and other food products. The main advantage of using *Pleurotus* spp to upgrade lignocelluloses waste is their selective degradation of lignin and hemicellulose, as a result of which the cellulose is exposed and can be utilized by ruminants. Utilization of these materials is clearly dependent on *Pleurotus*' ability to secrete a range of enzymes, including peroxidase, laccase, cellulase, hemicellulase and xylanase. One of the advantages of the *Pleurotus* group is their ability to grow on lignocellulosic substrates without the need for a composting or casing layer. *P. ostreatus* grown on wood sawdust (Giardina et al. 2000) and was found to exhibit manganese-independent activity.

2.5 Summary of Pretreatment

One technology that is efficient for a particular type of biomass materials might not work for another material. Various pretreatment processes for lignocellulosic biomass, advantages and disadvantages are summarized in Table 2.2 below. The choice of pretreatment technology used for a particular biomass depends on its composition and byproducts produced as a result of pretreatment. These factors will significantly affect the cost. The choice of pretreatment technology for a particular material depends on which components of the biomass material need to be altered (Parveen et al, 2009).

Table 2.2 Summary of various processes used for the pretreatment of lignocellulosic biomass

Pretreatment process	Advantages	Limitation and disadvantages
Mechanical comminution	Reduces cellulose crystallinity	Power consumption usually higher that inherent energy
Steam explosion	Causes hemicellulose degradation,lignin transformation and cost effective	Incomplete destruction of the lignin-carbohydrate matrix
AFEX	Increase accessible surface area, removes lignin and hemicelluloses	Not efficient for biomass with high lignin content
CO_2 explosion	Increase accessible surface area, cost effective and does not produce inhibitors for downstream process	Does not modify lignin and hemicelluloses
Ozonolysis	Reduce lignin content, does not produce toxic residue	Large amount of ozone required, expensive
Acid hydrolysis	Alter lignin structure	High cost, and equipment corrosion
Alkaline hydrolysis	Remove lignin and hemicellulose, increase accessible surface area	Long residence time required, irrecoverable salt form
Pyrolysis	Produces gas and liquid product	High temperature, ash production
Biological	Degrades lignin and hemicellulose, low energy requirement	Rate of hydrolysis is very low

Sources: Caixia & Yebo (2012)

2.6 Kinetic Study

According to Romero et al (2010) research, the model that best fit experimental data was proposed by (Saeman, 1945). Saeman's model considers a first order, irreversible reaction. Considering first order kinetic reaction for fiber hydrolysis, the following equation is derived:

$$\text{OPT (s)} \xrightarrow{\text{acid hydrolysis}} \text{glucose (aq)} \qquad (2.6.1)$$

$$-\frac{dFH}{dt} = K_{1.} F_H \longrightarrow \{t=0 \quad Y=Y_0, \quad F_H = F_{Hmax} \qquad (2.6.2)$$

$$\longrightarrow \{t=\infty \quad Y=Y_{max}, \quad F_H= 0$$

Taking into account that at each temperature assayed, there is a minimum sugar concentration at the beginning of residue hydrolysis and a maximum sugar concentration attainable at high process time, $Y_{max,}$ the sugar generation may be expressed by:

$$\frac{dY}{dT} = K_2(Y_{max} - Y) \longrightarrow \{t=0 \quad Y=Y_0, \qquad (2.6.3)$$

$$\longrightarrow \{t=\infty \quad Y=Y_{max},$$

Assuming that sugar generation is produced in several consecutive steps starting with fiber hydrolysis, Y_0 is the initial sugar concentration obtained following instantaneous hydrolysis of fiber and F_H is amount of

biomass (g). Using equation acid hydrolysis of hardwood to produce glucose as above, there are three kinetic parameters, which are K_1, K_2, Y_{max}. Where stated that K_1 is biomass degradation (min^{-1}), K_2 is a sugar generation rate (min^{-1}), Y_{max} is maximum concentration of glucose (g/l).

2.7 Fourth Order Runge-Kutta Method

This RK fourth order method will be use to solve ordinary differential equation in the kinetic study. This RK fourth order method also will be use to determine the kinetic parameters which are K_1, K_2 and Y_{max}. The most popular RK methods are fourth order. As with the second-order approaches there are an infinite number of versions. The following is the most commonly use form, and therefore called classical fourth order RK method:

$$y_{i+1} = y_i + 1/6 (k_1 + 2k_2 + 2k_3 + k_4) h \quad (2.7.1)$$

Where

$$K_1 = f (x_i , y_i) \quad (2.7.2)$$

$$K_2 = f (x_i + ½ h, y_i + ½ k_1 h) \quad (2.7.3)$$

$$K_3 = f (x_i + ½ h, y_i + ½ k_2 h) \quad (2.7.4)$$

$$K_4 = f (x_i + h, y_i + k_3 h) \quad (2.7.5)$$

Notice that for ODEs that are function of x alone, the classical fourth order RK method is similar to simpson's 1/3 rule. Each of the k's represents as slope. Equation 2.7.1 then represents a weighted average of these to arrive at the improved slope (Chapra & Canale, 2010).

Moldes et all (1999) study kinetic cogeneration of cellobiose and glucose from pretreated wood and bioconversion to lactic acid. Models for the generation of cellobiose and glucose were developed assuming two sequential reactions (conversion of cellulose into cellobiose and hydrolysis of cellobiose to glucose) with end-product that results in competitive inhibition. The models lead to high cellulose conversions and sugar concentrations. The set of two differential equations solved using a combination of a numerical integration of the equation system by a fourth-order Runge- Kutta method and an optimization algorithm based on Newton's method.

$$-\frac{dCl}{dt} = \frac{r_m.Cl}{k_m\left(1+\frac{C_b}{k_i}\right)+Cl} \tag{2.7.6}$$

$$\frac{dG}{dt} = \frac{r_m'.Cb}{k_m'\left(1+\frac{G}{k_i'}\right)+Cb} \tag{2.7.7}$$

Eq 2.7.6 above is the equation of cellulose hydrolysis rate whereas Eq 2.7.7 shows for glucose generation rate. According to the equations above and considering the initial substrate concentration, these kinetic parameters allowed the calculation of sugar concentrations. This research shows that ordinary differential equation can be solve using fourth-order Runge–Kutta method.

Kitakawa et al (1998) study kinetic model for oligosaccharide hydrolysis. K_m, V_{max} and α are the Michaelis-Menten type kinetic constants. These kinetic constants estimated by fitting the model with the experimental data obtained under various conditions. The new constant, α, is estimated at 0.411, and almost the same as the experimental values. The model has been extended for the immobilized enzyme system by taking into account the intraparticle mass transfer resistance. To obtain the calculated values under a certain set of constants, the simultaneous differential equations, eq. (2.7.8) until eq (2.7.11), numerically solved using the Runge-Kutta method. Time step for the numerical calculation is set at 60s. Hence, the kinetic model well simulates the experimental result for the system.

$$\frac{dC_6}{dt} = v_6 = \frac{-3\,V_{max}C_6}{K_m + 3C_6 + C_4} \tag{2.7.8}$$

$$\frac{dC_4}{dt} = v_4 = \frac{V_{max}[\,3\,(1-\alpha)C_6 - C_4}{K_m + 3C_6 + C_4} \tag{2.7.9}$$

$$\frac{dC_3}{dt} = v_3 = \frac{6V_{max}\alpha C_6}{K_m + 3C_6 + C_4} \tag{2.7.10}$$

$$\frac{dC_2}{dt} = v_2 = \frac{V_{max}[\,3\,(1-\alpha)C_6 + 2C_4}{K_m + 3C_6 + C_4} \tag{2.7.11}$$

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Process Flow Chart

The brief process method for this research is shown in the flow chart below. RK fourth order method will be utilized in the kinetic study to solve the ordinary differential equation. There are four major steps to be used for this research.

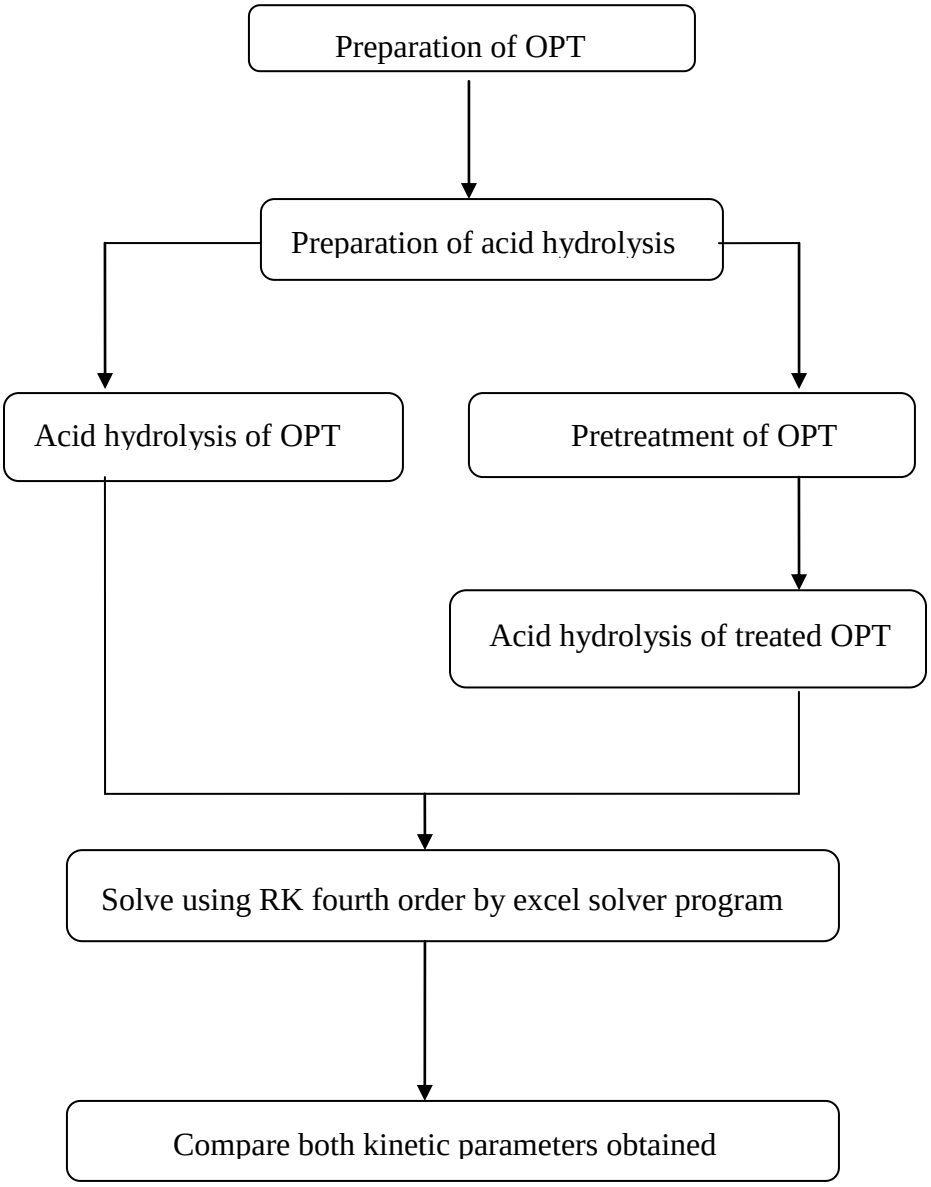

Figure 3.1 Simple flow chart of the process

In the preparation of OPT, the OPT will be cut into small pieces. After that, those OPT will be dried, weighted for 5g and prepared for acid hydrolysis. In acid hydrolysis preparation, two stage of acid hydrolysis will be applied and acid that will be use is sulfuric acid. First stage used

60% acid concentration whereas second stage used 30% acid concentration. First method is OPT without pretreatment. In this method, acid hydrolysis will be applied directly to OPT. First, OPT will be submerged in 60% sulfuric acid for 30 min and respectively at 60°C. Secondly, OPT will be applied with 30% acid hydrolysis for 60 min and respectively at 80°C. After that, OPT that have been diluted with sulfuric acid will be filtered. Thus, OPT solution will be obtained. Then, this filtrate will be neutralized with NaOH and will be ready for analyze of glucose.

The second method is OPT with pretreatment of acid hydrolysis. This can be done by biodelignification process. This biodelignification uses a type of commercialized local fungi, *pleurotus ostreatus* and can also be known as oyster mushroom as the agent of biodelignification. According to Zulsyazwan (2012) during the growth of the fungi, experiment will be conducted at these following conditions which are 25.16°C, pH 7.54, 2.38 mL/12 hour for moisture content and 1:2 fungal to medium ratio. After that, acid hydrolysis process will be applied and analyzed for glucose production.

After both samples are analyzed, two of kinetic acid hydrolysis will be obtained. One is kinetic acid hydrolysis without pretreatment and another one is kinetic acid hydrolysis with pretreatment which is biodelignification.

$$-\frac{dFH}{dt} = K_{1.} F_H \longrightarrow \{t=0 \quad Y=Y_0, \quad F_H = F_{Hmax}$$

$$\longrightarrow \{t=\infty \quad Y=Y_{max}, \quad F_H = 0 \qquad (3.1)$$

$$\frac{dY}{dT} = K_2(Y_{max} - Y) \longrightarrow \{t=0 \quad Y=Y_0,$$

$$\longrightarrow \{t=\infty \quad Y=Y_{max},$$

$$= K_2 (Y_{max} - Y) \qquad (3.2)$$

Using equation acid hydrolysis of hardwood to obtain glucose, there are three kinetic parameters obtained which are K_1, K_2 and Y_{max}. Where stated that K_1 is biomass degradation (min^{-1}), K_2 is a sugar generation rate (min^{-1}) and Y_{max} is concentration of glucose (g/l). By using equation (3.1), at the beginning of experiment and the value of $Y_{max,}$ the amount of hydrolyzed fiber which generates sugar is determined at each trial. Considering that equation (3.1) cannot predict the amount of fiber that is instantaneously hydrolyzed, this is calculated from Y_0 values. Hence, kinetic parameters can be solve using RK fourth order method by excel solver. The Y_{max} is correlated with F_H and determination coefficient, R^2 will be determined.

With this equation, a table with variables t, F_H, and Y can be created. F_H is the mass of raw material (OPT), t is the reaction time of acid hydrolysis and Y is sugar concentration produced. Y value will be plotted in the graph to get R^2. Kinetic parameters can be solve using RK fourth order method by excel solver. The Y_{max} is correlated with F_H and determination coefficient, R^2 will be determined. Thus, kinetic model obtained will prove to be useful for further technical and economic studies. This can be achieved for example to maximized quantity of glucose by referring to this kinetic parameters obtained.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Determination of Kinetic Parameter

In this research, two kinetic of acid hydrolysis must be obtained. One kinetic is for acid hydrolysis untreated oil palm trunk (OPT) and another one is for treated OPT. To determine the kinetic parameters of this research, there are two major steps involved. Firstly, the collection of the experimental data which involved the glucose concentration and biomass of OPT left after experiment. Second step is regarding kinetic modeling part. Based on the experimental data and from developed kinetic model, the best fit kinetic parameters were determined.

The process of acid hydrolysis of OPT in this experiment was modeled by first order reaction kinetic. The kinetic model was obtained from previous researcher were study acid hydrolysis of hardwood. This

model expected to predict glucose concentrations for further use. Based on the experiment conducted, there are three kinetic parameters constant which are Y_{max} (maximum sugar concentration attainable at high process time), K_1 (biomass decomposition rate) and K_2 (sugar release rate).

In this work, the model that best fit experimental data was that proposed by Saeman (1945), which initially derived for cellulose hydrolysis but also applied by other researcher to hemicellulose fraction from agricultural residue (Tellez-Luis et al, 2002). Saeman's model considers a first order, irreversible reaction. According to Romero et al (2010), the sugar generation may be expressed by:

$$\frac{dFH}{dt} = -K_{1.} F_H \longrightarrow \{t=0 \quad Y=Y_0, \quad F_H = F_{Hmax} \longrightarrow \{t=\infty \quad Y=Y_{max}, \quad F_H = 0 \qquad (4.1)$$

Where:

K_1 = biomass decomposition rate (min^{-1})

F_H = biomass of OPT (g)

$$\frac{dy}{dt} = K_2(Y_{max} - y) \longrightarrow \{t = 0 \quad Y = 0 \longrightarrow \{t = max \quad Y = Y_{max} \qquad (4.2)$$

Where:

K_2 = sugar release rate (min^{-1})

Y = sugar concentration obtained

Y_{max} = maximum sugar concentration obtained

4.2 Biomass Degradation

Figure 4.1 is the graph of biomass versus time for untreated OPT. This study not focuses only on kinetic parameter of glucose production but also biomass degradation. From the graph, it can be observed that mass decline as the time increase. This because as the experiment run, the more OPT mass has been hydrolyzed. From time 10-30 minutes, the mass of OPT have been decreased gradually as high acid concentration was used whereas from time 30-90 minutes, the mass of OPT decreased slightly as the low concentration of acid was used.

According to Barneto et al (2010), the rapid mass loss at the beginning of experiment associate with holocellulosic fraction (cellulose and hemicellulose). On the other hand, slow mass loss at the end of experiment is because of lignin fraction. This fact could be consequence of the degradation of polymeric chains of carbohydrates. Composting affect hemicellulose and cellulose fractions, breaking polysaccharides chains and making easier acid hydrolysis take place. At the end of

experiment, it can be seen that rate of biomass decomposition become slower and it is close to finalize.

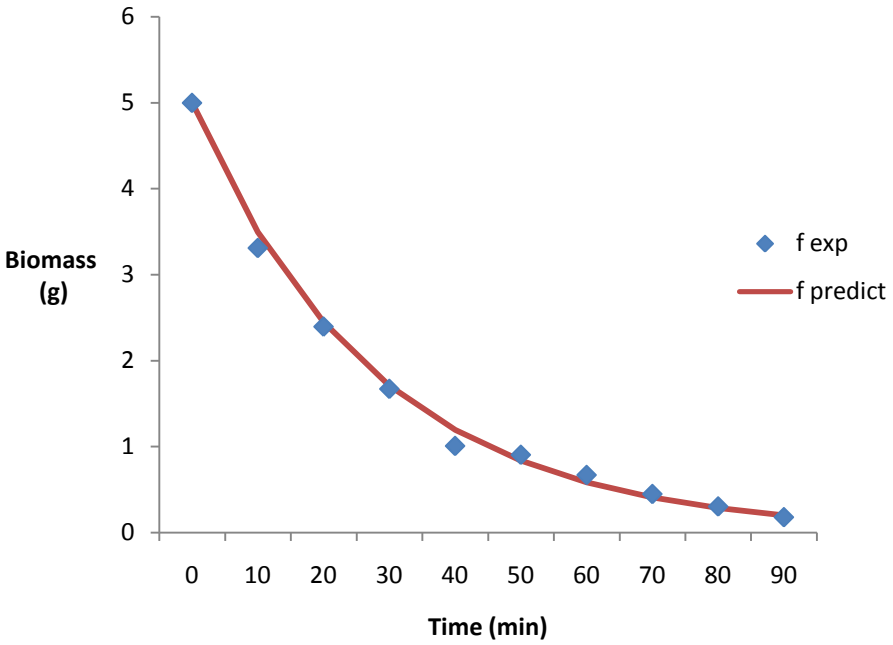

Figure 4.1 Graph of Biomass versus Time for Untreated OPT

4.3 Glucose Production

In this study, glucose is the main byproduct released during hydrolysis of OPT with sulfuric acid. After done with all of the experiments, the data collected were analyzed by using RK fourth order (analysis of differential equation) in excel solver, as the experimental done, here are the result for acid hydrolysis of untreated OPT. Figure 4.2 below is the graph of concentration of glucose versus time for untreated

OPT. At time zero, the concentration of glucose is assumed to be zero because no lignin has been degrade at this time. In this study two stage of acid hydrolysis was used. At time 10- 30 minutes, it can be seen that the glucose concentration increased gradually. At time 10-30, the OPT was hydrolyzed with 60% H_2SO_4 acid concentration at 60°C. Meanwhile, for experiment at time 40-90 minutes, the acid concentration used is 30% respectively at 80°C. At time 30-70 minutes, glucose concentration can observed to increase slowly and at time 70-90 minutes, the glucose concentration become steady and started to decline a little bit. Overall, it can be said that the concentration of glucose increased gradually with time and reached a constant value within 70 minutes. A slight decrease was also observed in the glucose concentration over a long time. This fact suggests that decomposition reactions can exist, for example to hydroxymethylfurfural (HMF). HMF is naturally generated in sugar-containing food during heat treatments. From glucose, the reaction can lead to decomposition products, mainly HMF. This is formed in acid medium due to the release of 3 molecules of water from hexose. In this experiment HMF might be exist in the acid hydrolysis process while the OPT is heat up at 80°C. From graph below, it can be observed that the blue line is the expected result obtained from kinetic study whereas the red line is from experiment.

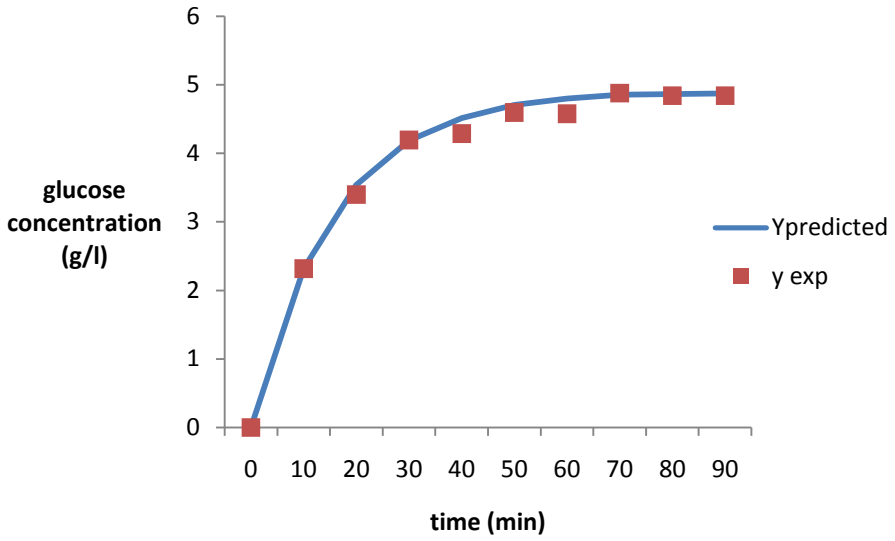

Figure 4.2 Graph of Glucose Concentration versus Time for Untreated OPT

In 1983, Stinson published a paper in which he described that two stage hydrolysis have been conducted in the past to separate hydrolysis of hemicelluloses and cellulose fraction present in woody biomass. This process can be very effective in the utilization of low grade biomass. In this study only 5g of biomass of OPT is used. Moreover, by using two stage of acid hydrolysis, any hemicellulose that was not converted in the first step could be converted further in second hydrolysis step. In this experiment, the first stage of acid concentration which is 60% H_2SO_4, glucose production increase gradually and rapidly. Chin et al (2011) states that in the first stage, strong acid concentration is used because strong acid concentration in the first stage serves to break the bonds that hold cellulose in the crystalline state and caused the extreme swelling or dissolving of the cellulose. Xiang et al (2004) had reported that

hydrolysis rate increases gradually with respect to acid concentration. Indeed, Mathewson (1980) has reported that prolonging the reaction time during hydrolysis with higher acid concentration will cause degradation of the decrystalized cellulose at high acid concentration. This is the reason only 30 minutes reaction time is used in this experiment with high concentration of acid.

For second stage hydrolysis, the sugar production increase slightly and slowly. At this stage, the decrystalized cellulose is mainly hydrolyze into glucose and forms a homogeneous gelatin with the acid. At this point, the cellulose is believes to be extremely susceptible to hydrolysis. Thus, any weak acid concentration at modest temperatures can easily hydrolyze the decrystalized cellulose to with little degradation (Chin et al, 2011). The reason for two temperatures was used because in 1986, Aravamuthan et al demonstrated dilute acid hydrolysis of hemicellulose at low temperature followed by hydrolysis of cellulose at high temperature up to 240°C and the conversion of cellulose is believed to achieve approximately 50% under these conditions. However, hydrolysis equipment must be designed to withstand corrosive conditions at high temperature and this will involve high cost.

The mechanism of the hydrolysis reaction includes (Carrasco, 1991; Fengel & Wegener, 1984; Harris, 1952): (i) diffusion of protons through the wet lignocellulosic matrix; (ii) protonation of the oxygen of a heterocyclic ether bond between the sugar monomers; (iii) breaking of the ether bond; (iv) generation of a carbocation as intermediate; (v) solvation of the carbocation with water; (vi) regeneration of the proton with cogeneration of the sugar monomer, oligomer or polymer depending on the position of the ether bond; (vii) diffusion of the reaction products in the liquid phase if it is permit for their form and size.

4.4 Kinetic Parameters

Values obtained by previous researchers on kinetic study were tabulated in Table 4.1 and Table 4.2 for comparison. The reaction constants K_1 and K_2 are determined using the solver function on Microsoft Excel. By minimizing the sum of the square of the error between the experimental data and the model data obtained, accurate reaction parameters can be found. The solver function operates by attempting to acquire a value of zero error through changing of the K_1 and K_2 values. Higher value of K_2 required shorter residence time to obtain high recovery of glucose in the resulting hemicellulosic hydrolysate (Rafiqul & Mimi, 2012). R^2 showed a good agreement between experiment and predicted data.

Table 4.1 presents the results obtained from the analysis of kinetic parameter of biomass decomposition rate. K_1 means amount of hydrolysed biomass which generates sugar. From the above table, Aguilar et al (2002) and Jeong et al (2011) study shown that kinetic parameter of decomposition reactions (K_1) are small or almost 0. It can be supposed that this is due to the high activation energy of the reactions of glucose degradation (Aguilar et al 2002). This might be because the raw material use is sorghum straw and they study glucose as a secondary product obtained in the hydrolysis. Other than that, Arastehnodeh et al (2012) obtained lower value of decomposition rate than this study. This supposed that Arastehnodeh et al (2012) used walnut green skin as raw material. However, value of R^2 is higher than other research stated, the statistical parameter (R^2) show a good agreement between experimental data and experimental data. However this study only focused on glucose production. This study shows the highest biomass decomposition because this study only focused on glucose production and the concentration of acid used is in good range. Hence, biomass left almost zero.

Table 4.1 Kinetic parameter of OPT decomposition rate

Researcher	$K_1(min^{-1})$	R^2
This study (2012)	0.011454	0.992
Arastehnodeh et al (2012)	0.00280	0.98
Aguilar et al (2002)	0.00029	0.960
Jeong et al (2011)	1.043×10^7	-

According to Sherban & Idayu (2012), OPT contains holocellulose, hemicelluloses and lignin of almost 73.06%, 34.1% and 24.7% respectively, which cellulose and hemicellulose can be converted to reducing sugar by hydrolysis. The table below shows the kinetic parameter of sugar release rate for various study included this study. Hence, the sugar release rate is depending on efficiency of acid hydrolysis. Other than that, factors affecting the hydrolysis of cellulose include porosity (accessible surface area) of the biomass materials, cellulose fiber crystallinity, and content of both lignin and hemicellulose. Furthermore, cellulose hydrolysis strongly depends on its degree of crystallinity and swelling state (Xiang et al., 2004). As the above shown that the kinetic value of Aguilar et al (2002) shown the highest kinetic value compare to Rafiqul & Mimi (2012), and this research. However, the glucose yield is lower than this study. The rate of cellulose degradation

by acid hydrolysis is depending on concentration of acid, reaction times, temperature used and types of raw material.

According to Balat (2009) cellulose and hemicelluloses contents are more in hardwoods (78.8%) than softwoods (70.3%). That is why the kinetic parameter of this study is lower than Aguilar et al (2012) and Rafiqul & Mimi (2012) because this research use hardwood as raw material compared to other research that use softwood as raw material. Besides that, cellulose content in palm oil trunk is 73.06% compares to sorghum straw: 35%, sugar cane basesses: 38.9%, and rice straw: 43.4% (Ghasem et al, 2007). Consequently, the rate of sugar production is slightly slower in this experiment because the cellulose content of palm oil tree is the highest and it takes time for the acid hydrolysis to degrade cellulose into sugar.

In addition, Y_{max} value in this study shows the highest result compared to other research stated above. One of the reason is the cellulose content in hardwood is higher than softwood. This study use hardwood as the raw material which absolutely content higher glucose content than softwood. NG et al (2011) stated that dilute acid hydrolysis is used to extract xylose whereas concentrated acid hydrolysis to obtain glucose. From Table 4.2 below, only this study use a high concentration of acid because the aim is to obtain glucose whereas the other studies

stated in the Table 4.2 use lower concentration of acid as the aim is to obtain xylose.

According to Aguilar et al (2002), Y_{max} obtained from their study is 3g/l. The optimum condition is stated as 2% concentration acid used at 122°C for 24 minutes reaction time. In Aguilar et al (2002) study, glucose is only a by-product obtained in the acid hydrolysis of sugar cane bagasse. The glucose from cellulose is not usually hydrolysed in the range of operational conditions commonly used for the acid hydrolysis. Therefore, it is probable that the released glucose proceed almost quantitatively from hemicellulose. The low concentration of glucose indicated that the cellulose fraction remained almost unchanged during acid hydrolysis and thus the glucose probably came from the hemicelluloses. A lower Y_{max} value and glucose yield in their study compared to this study indicating a small degradation of the cellulosic fraction.

According to Rafiqul & Mimi (2012), the raw material used is Meranti wood saw dust and the optimum conditions in their study are 6% acid used at 130°C for 120 minutes reaction time. The result for Y_{max} is 4.2 g/l and the value of K_2 is 0.03006 min^{-1} which is higher than this study with R^2; 0.992 suggesting that the model adequacy to be reasonably accurate and reliable. Their study shows a very good agreement with

expected data. The aim production of their study is xylose and at the same time, other byproducts such as glucose, furfural, acetic acid, etc., were also generated in low amounts during hydrolysis. During hydrolysis, acid concentration was found to be the most important parameter affecting the sugar yield (Neureiter et al., 2002). This is the reason why the glucose yield is lower than this study because the acid concentration used is only 6%.

Table 4.2 Kinetic parameter of sugar release rate

Researcher	K_2 (min^{-1})	R^2	Y_{max} (g/l)	Yield (g glucose /g biomass)
This study (2012)	0.015036	0.9232	4.878	44.2
Aguilar et al (2002)	0.0357	0.979	3.0	36.5
Rafiqul & Mimi (2012)	0.03006	0.9992	4.2	33.6

4.4 Biodelignification as Pretreatment of Acid Hydrolysis

Biodelignification is importance as the microorganism is use to treat lignin and in the same time enhance enzymatic hydrolysis. As for this research, a local type of fungi, *Pleurotus ostreatus* or commercially known as “Oyster Mushrooms”, were used as an agent of delignification.

This fungus is able to produce laccase and manganese peroxidase (MnP) isoenzymes, hemicellulases and a poor complex of cellulases. According to Zulsyazwan, 2012 during the growth of the fungi, the optimum conditions are as follows:

Table 4.3 Table of optimum conditions of *pleurotus ostreatus*

Parameter	Value
Temperature	25.16°C
Ph value	7.54
Moisture content	2.38 ml/12 hour
Fungi to medium ratio	1:2 ratios

There are four factors for optimum condition of fungi to grow in biodelignification process. Most wood destroying fungi grow best and their growth curves show well-defined maximum temperature from 20-35°C (Ungger, 1995). According to a research done by Buah et al. (2010), the growth of oyster mushroom requires high temperature (25-30 °C) for the vegetative growth called spawn running and lower temperature (18-25 °C) for fruit body formation (Viziteu, 2000). The susceptibility of wood to attack by fungi also depends on its hydrogen ion concentration (pH value). From Chang and Miles (2004), pH optimum for the mycelial growth of *Pleurotus ostreatus* is between 4-6, while Gyurko (1979) determined the pH optimum to 5, 5.5, and 8. Besides that, fungi need

optimum water content to degrade the lignin and referring to a research by Mahler (1992) the moisture content should be maintained between 80-90% for the mycelia growth. For fungi to medium ratio factor, as in a book written by Steven (1981), it stated that a wide range of media are used for growing fungi. Fungi need a wide media for sporulation process and enhance the degradation of the lignin.

Graph 4.3 below shows graph of biomass versus time for treated OPT. As time increase, less biomass of OPT left. Biomass of OPT left for untreated is 0.18 g which is higher compared to treated OPT biomass which is 0.06112g. This is because as time increase, for treated OPT, *pleurotus ostreatus* able to produce laccase, manganese peroxidase (MnP) isoenzymes, hemicellulases and a poor complex of cellulases that can cause a swelling and softening of OPT cell walls as a result of the modification and depolymerization of the lignin (Scott, 2001). Thus, increases the biomass of OPT have been hydrolyzed compared to untreated OPT. Figure 4.3 indicates that final biomass left nearly approaches zero, suggested that acid hydrolysis succeeded in degrade the lignin of OPT in the same time increase the yield of sugar obtained. According to Ghasem (2007) in most lignocellulosic wastes, due to the presence of cellulose crystallinity, the chemical attack on the cellulose is retarded. Therefore, pretreatment was necessary to increase the susceptibility of lignocellulose for hydrolysis reaction. From the result

obtain, biodelignification as pretreatment to acid hydrolysis can accelerate the rate of reaction and the extent of cellulose hydrolysis.

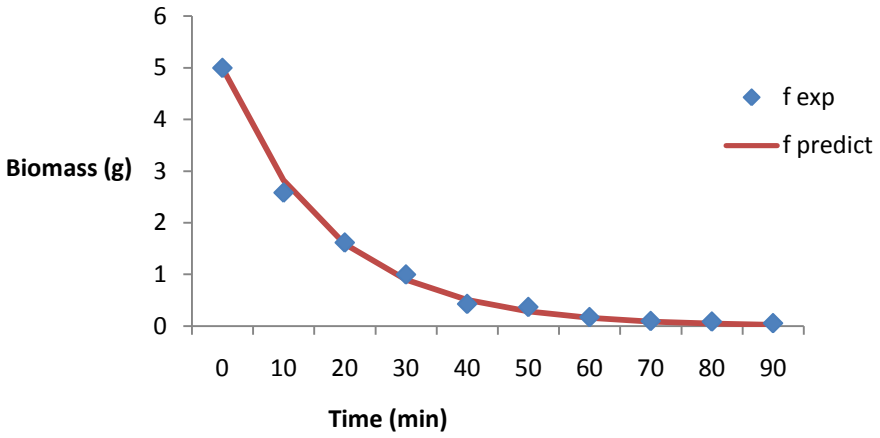

Figure 4.3 Graph of biomass OPT versus time for treated OPT

Graph of 4.4 below shows the intercorrelation among the nine measures of glucose concentration versus time for treated OPT. The graph shows that the highest production of glucose obtained is 5.266 g/l. Glucose production of treated OPT shows increment with 7.95% from the untreated OPT. This prove that biodelignification can be applied as pretreatment to acid hydrolysis with the reason that biodelignification can help to break down lignin first before acid hydrolysis applied. Hence, higher glucose production can be obtained. White rot fungi included *pleurotus ostreatus* is the most efficient ligninolytic organisms described to date. Their ability to degrade lignin and a wide variety of aromatic compounds is due to a nonspecific extracellular enzyme system, involves

lignin peroxidases, laccases and manganese-dependent peroxidases as well as hydrogen-producing oxidaseslaccase. Production of ligninolytic enzymes in the white rot fungi is influenced by culture conditions, the composition of the medium and optimum levels for these factors vary according to microorganism and substrate (Zhao et al, 1996). Although lignin-degrading microorganisms occur naturally in composts, (Tuomela et al., 2000), it has been shown that inoculation with lignin-degrading microorganisms accelerates the composting process and increase the compost quality (Lopez et al, 2006). White rot fungi degrade lignin by means of oxidative enzymes and lignin is mineralized to CO_2 (Huang et al, 2008).

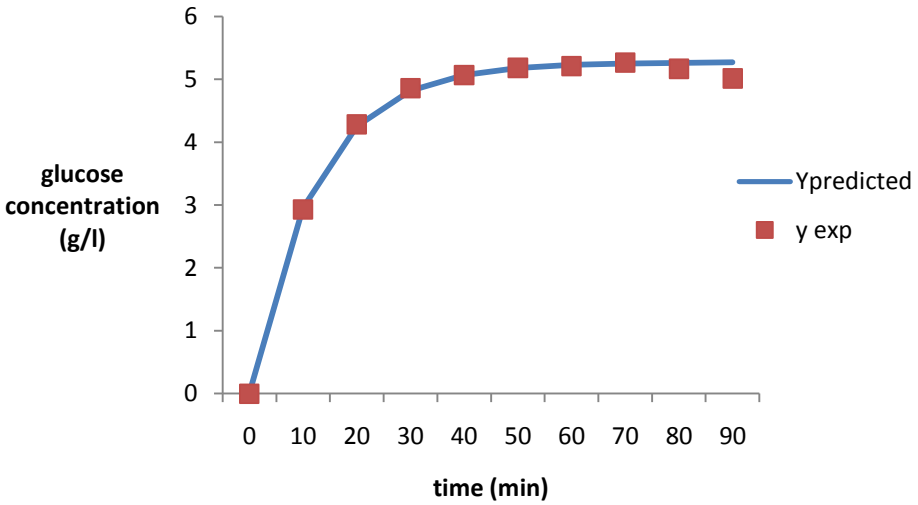

Figure 4.4 Graph of glucose concentration versus time for treated OPT

4.5 Comparison of Kinetic Parameters of Pretreatment Acid Hydrolysis

Table 4.4 below shows the comparison of the treated and untreated OPT. From the kinetic study the value of predicted data also obtained. The comparison data for untreated and treated OPT as follow:

Table 4.4 Table of Comparison Data of Treated and Untreated OPT

Untreated OPT	Treated OPT
K_1 = 0.007774 min^{-1}	K_1 = 0.011454 min^{-1}
Error = 0.087399	Error = 0.083729
Y_{max} = 4.878 g/l	Y_{max} = 5.266 g/l
K_2 = 0.012584 min^{-1}	K_2 = 0.015036 min^{-1}
Error =0.130249	Error = 0.076811

It can be seen from the data in Table 4.4 that treated OPT obtain higher sugar release rate and OPT decomposition rate than untreated OPT. The data in Table 4.4 demonstrate that the biomass decomposition K_2 and rate of sugar formation K_1 increased with biodelignification as pretreatment to acid hydrolysis is applied. It was also noticed that the value of treated OPT K_1 is about 47.3% higher than the untreated OPT K_1 value while the value of treated OPT K_2 is about 19.5% higher than the untreated OPT K_2 value. These findings suggested that the treated OPT

required a shorter residence time to obtain the highest recovery of glucose resulting hemicellulosic hydrolysate and high rate of biomass decomposition to glucose. Meanwhile, highest glucose production (Y_{max}) of treated OPT also shows higher than untreated OPT by 8%. Higher Y_{max} value of treated OPT is the benchmark of successful of this study because the main production in this study is glucose. Since, further application of this study is to scale up the glucose production from OPT by using and referring to this kinetic study. The percentage errors were also well within acceptable value (5%), suggesting that the model adequacy to be reasonably accurate and reliable. Meanwhile, error of treated OPT also less, resulting more accurate result than untreated OPT. These prove that, biodelignification as pretreatment to acid hydrolysis help to enhance acid hydrolysis.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In conclusion, the objective of this experiment is to study the kinetic acid hydrolysis of OPT to produce glucose is successfully achieved. Biodelignification was choose as pretreatment to acid hydrolysis. In the biodelignification process, *pleurotus ostreatus* is used as it is able to produce lignolytic and cellulolytic enzymes that importance in breakdown the lignin of OPT. RK^{4th} order and excel solver was used in modeling, analyzing and finding the kinetic parameters for kinetic study. From the kinetic studies, the kinetic parameters value of untreated OPT are as follow: K_1 = 0.007774 min^{-1}, Y_{max} = 4.878 g/l and K_2= 0.012584 min^{-1} while kinetic parameters value of treated OPT are as follow: K_1= 0.011454 min^{-1}, Y_{max} = 5.266 g/l and K_2= 0.015036 min^{-1}. This demonstrated that the rate of biomass decomposition K_1 and sugar

formation K_2 increased with biodelignification as pretreatment to acid hydrolysis is applied. These findings suggested that the treated OPT required a shorter residence time to obtain the highest recovery of glucose, the highest OPT degradation to glucose and lastly the higher glucose yield than untreated OPT. This is because high value of K_1 means high rate to degrade biomass to produce glucose, high value of K_2 shows the faster time needed to achieve glucose production and high value of Y_{max} shows high glucose production obtained. For comparison with other researcher, kinetic parameter of biomass decomposition rate (K_1). Aguilar et al (2002) and Jeong et al (2011) study shown that K_1 are small which are 0.00029 and 1.043×10^7 respectively. Consequently, the rate of sugar production (K_2) is slightly slower in this experiment than Aguilar et al (2012) and Rafiqul & Mimi (2012) because the cellulose content of palm oil tree is the highest which means it takes time to degrade to sugar. For kinetic parameter Y_{max}, the glucose yield of Rafiqul & Mimi (2012) is lower than this study which is 4.2 g/l.

5.2 RECOMMENDATION

As we know, carbohydrate is sugar molecule linked together. Sugar can act as a renewable resource and derived from a variety of biological feedstock such as lignocellulosic biomass thus can be further converted to a number of high-value bio-based fuels. Besides that, oil palm trunk interest of researcher as a resource of starch and other sugar, this study recommended that future research study about kinetic acid hydrolysis of OPT for production of xylose. As xylose is a hemicellulosic sugar which can be an economical starting raw material for the production of a wide variety of compounds or fuels by chemical and biotechnological processes. Furthermore, this kinetic study can also apply not only to oil palm trunk but to other hardwoods raw materials. Moreover, this kinetic study can be used for further technical and economic studies as kinetic parameters obtained can be applied to maximized glucose production in industry.

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