Project Code :
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A PROJECT DETAILS

i **Title of Research:**
Production of Second Generation Bioethanol—Effect of various pre-treatments on non-wood biomass towards sugar production

ii **Account Number:** 1001/PTEKIND/814164

iii **Name of Research Leader:** Assoc. Prof. Dr. Leh Cheu Peng

iv **Name of Co-Researcher:**

1. Prof. Dr. Lee Keat Teong
2. Assoc. Prof. Wan Nadiah Wan Abdullah

v **Duration of this research:**

a) **Start Date** : ...... **15/7/2012**............
b) **Completion Date** : ....**14/7/2015**...........
c) **Duration** : ....**36 months**........ + 12 month
d) **Revised Date** (*if any*) : ....**14/7/2016**...........

B ABSTRACT OF RESEARCH

(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English. This abstract will be included in the Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Abstract
Water, acid and alkaline pretreatments prior to enzymatic hydrolysis of the three fibres for sugar production were optimized in this study. Response Surface Methodology was applied to statistically analyze and optimize pretreatments conditions based on total glucose yield as response variable. The effects of chemical composition and physical structure of the fibre on the total glucose yield were discussed comprehensively. Among the fibres, alkaline pretreated kapok fibre achieved the highest total glucose yield (39.6%), followed by the water pretreated EFB fibre (38.1%) and kenaf core fibre (25.5%). Alkaline pretreated kapok fibre exhibited the highest total glucose yield, mainly due to its high tendency on lignin elimination and the most severe structural destructive effect. In contrast, water pretreatment was the most effective method in enhancing the enzymatic hydrolysability of EFB and kenaf core fibres due to the removal of hemicellulose in a large amount (>77%). However, unlike EFB fibre, kenaf core fibre did not show better enzymatic hydrolysability after pretreatment, which

4.	Refrigerator	2779.00	Lab 229, School of Industrial Technology	good

 # Please attach the Asset/Inventory Return Form (Borang Penyerahan Aset/Inventori) – Appendix 1

D RESEARCH ACHIEVEMENTS

i **Project Objectives** *(as stated/approved in the project proposal)*

No.	Project Objectives	Achievement
1	To study the effect of acidic hydrolysis pretreatments on chemical composition of various biomass.	Achieved
2	To investigate the potential of oxygen delignification treatment based on chemical composition of various biomass.	Achieved
3	To study the feasibility and effectiveness of various pretreated lignocelluloses biomass for the production of ethanol.	Achieved
4	To study the effect of different pulping methods on acidic pretreated lignocelluloses biomass.	Withdrawn as only pre-treatment already achieved maximum glucose production
5	To optimize processes' parameters through design of experiment.	Achieved

ii Research Output

a) Publications in ISI Web of Science/Scopus

No.	**Publication** (authors,title,journal,year,volume,pages,etc.)	**Status of Publication** (published/accepted/ under review)
1.	Tye Ying Ying, Lee Keat Teong, Wan Nadiah Wan Abdullah, **Leh Cheu Peng.** Parametric optimization and comparison study of various pretreatments prior enzymatic saccharification of kenaf core (Hibiscus cannabinus) fiber for sugar production. *Renewable Energy* **99**: 205-215 (**2016**).	Published
2.	Tye Ying Ying, Lee Keat Teong, Wan Nadiah Wan Abdullah, **Leh Cheu Peng.** The world availability of non-wood lignocellulosic biomass for the production of cellulosic ethanol and potential pretreatments for the enhancement of enzymatic saccharification. *Renewable and Sustainable Energy Review* **60**: 155-172 (**2016**).	Published
3.	Tye Ying Ying, Lee Keat Teong, Wan Nadiah Wan Abdullah, **Leh Cheu Peng.** Effects of process parameters of various pretreatments on enzymatic hydrolysability of *Ceiba pentandra* (L.) Gaertn. (Kapok) fibre: A response surface methodology study. *Biomass and Bioenergy* **75**: 301-313 (**2015**).	Published
4.	Tye Ying Ying, Lee Keat Teong, Wan Nadiah Wan Abdullah, **Leh Cheu Peng.**	Published

	resource for second generation bioethanol: Parametric optimization and comparative study of various pretreatments prior enzymatic saccharification for sugar production, *Bioresource Technology* **140**:10-14 (**2013**).	
5.	Tye Ying Ying, **Leh Cheu Peng** Combination of aqueous pre-treatments with oxygen-alkali extraction for the enhancement of kenaf core fibre hydrolysability into fermentable sugars. *International Journal of Oil, Gas and Coal Technology.*	Accepted
6.	Tye Ying Ying, Lee Keat Teong, Wan Nadiah Wan Abdullah, **Leh Cheu Peng.** Total glucose yield as the single response in optimizing pretreatments for *Elaeis guineenses* fibre enzymatic hydrolysis and its relationship with chemical composition of fibre.	Under review

b) Publications in Other Journals

No.	**Publication** (authors,title,journal,year,volume,pages,etc.)	**Status of Publication** (published/accepted/ under review)
1.	Tye Ying Ying, Lee Keat Teong, WanNadiah Wan Abdullah, **Leh Cheu Peng.** The Effect of Various Pretreatment Methods on Oil Palm Empty Fruit Bunch (EFB) and Kenaf Core Fibers for Sugar Production. *Procedia Environmental Sciences* **20**:328-335 **(2014).**	Published

c) Other Publications
(book,chapters in book,monograph,magazine,etc.)

No.	**Publication** (authors,title,journal,year,volume,pages,etc.)	**Status of Publication** (published/accepted/ under review)

d) Conference Proceeding

No.	**Conference** (conference name,date,place)	**Title of Abstract/Article**	**Level** (International/National)
1.	The 4th International Conference Sustainable Future for Human Security, 18-21 October 2013, Kyoto, Japan	The effect of various pretreatment methods on kenaf core and oil palm empty fruit bunch (EFB) for sugar production.	International
2.	International Bioenergy (Shanghai) Exbition and Asian Bioenergy Conference 2015, 21-23 October 2015, Shanghai, P.R. China.	Aqueous Pretreatments as a key process to enhance the hydrolysability of kenaf core fibre for sugar production.	International

 # Please attach a full copy of the publication/proceeding listed above

iii **Other Research Ouput/Impact From This Project**
(patent, products, awards, copyright, external grant, networking, etc.)

The student (Miss Tye Ying Ying) was awarded “Best Paper” during the **The 4th international conference**

E HUMAN CAPITAL DEVELOPMENT

a) Graduated Human Capital

Student	Nationality (No.)		Name
	National	International	
PhD	1		1.Tye Ying Ying 2.
MSc			1. 2.
Undergraduate			1. 2.

b) On-going Human Capital

Student	Nationality (No.)		Name
	National	International	
PhD		1	1. Ajijolakewu Kamoldeen Abiodun 2.
MSc			1. 2.
Undergraduate			1. 2.

c) Others Human Capital

Student	Nationality (No.)		Name
	National	International	
Post Doctoral Fellow	1		1. Tye Ying Ying 2.
Research Officer			1. 2.
Research Assistant			1. 2.
Others (....................)			1. 2.

F COMPREHENSIVE TECHNICAL REPORT

Applicants are required to prepare a comprehensive technical report explaining the project. The following format should be used (this report must be attached separately):

- Introduction
- Objectives
- Methods

	• Discussion • Conclusion and Suggestion • Acknowledgements • References

G	**PROBLEMS/CONSTRAINTS/CHALLENGES IF ANY**
	(Please provide issues arising from the project and how they were resolved) The only one problem faced was only the common research problem—unexpected result for kenaf core pretreatment. This biomass couldn't acheive high glucose yeild as expected as kapok and EFB after simple pretreatment. Thus the project was extended to further the treatment study by having two-stage pre-treatment toward kenaf core viz. various aqueaus pretreatment followed by oxygen-alkaline extraction. This approach successfully improved the glucose yield up to 31%.
H	**RECOMMENDATION**
	(Please provide recommendations that can be used to improve the delivery of information, grant management, guidelines and policy, etc.) This research findings provide basic and comprehensive information related biomass pretreatment for cellulosic ethanol production or even other biomass related products.

Project Leader's Signature:

....

Name : Leh Cheu Peng

Date : 22 September 2016

DR. LEH C[illegible]
Associate Professor
School of Industrial Technology
Universiti Sains Malaysia

I COMMENTS, IF ANY/ENDORSEMENT BY PTJ'S RESEARCH COMMITTEE

- A very good research outcomes. Five publications (published + accepted) in citation indexed journal.
- Human capital – 2 PhD (National + International)

Signature and Stamp of Chairperson of PTJ's Evaluation Committee

PROFESOR DR. NORLI ISMAIL
Timbalan Dekan
(Penyelidikan, Siswazah Dan Jaringan)
Pusat Pengajian Teknologi Industri
Universiti Sains Malaysia
11800 Pulau Pinang Malaysia

Name :

Date : 27/9/16

b/p

Signature and Stamp of Dean/ Director of PTJ

Name :

Date : 28/9/16

PROFESOR DR. AZHAR MAT EASA
Dekan

Production of Second Generation Bioethanol—Effect of various pre-treatments on non-wood biomass towards sugar production

Comprehensive Technical report for RUI grant

(15 July 2012 – 14 July 2016)

Research Platform: PELANTAR KEJUTUTERAAN DAN TEKNOLOGI

Project Leader:	Associate Professor Dr. Leh Cheu Peng (School of Industrial Technology)
Project Member:	Professor Dr. Lee Keat Teong (School of Chemical Engineering)
	Associate Professor Wan Nadiah Wan Abdullah (School of Industrial Technology)

1 INTRODUCTION

1.1 General

Ethanol (ethyl alcohol or EtOH) is an attractive alternative fuel as it is a renewable bio-based resource. Due to it is an oxygenated, it can reduce particulate emissions in compression-ignition engines. Ethanol can produce from different kind of raw material. Ethanol feedstock can be classified into three types: (i) sucrose-containing feedstocks (e.g. sugarcane, sugar beet), (ii) starchy material (e.g. corn, wheat, barley) and (iii) lignocelluloses material (e.g. sugarcane bagasse, wood and straw) (Dias et al., 2009; Balat et al., 2008; Demirbas et al., 2009). Bioethanol converted from edible source is called first generation bioethanol (FGB) whereas second generation bioethanol (SGB) is produced from non-edible sources such as lignocelluloses biomass. Recently research on production of SGB are widely been investigated because it is the most abundant reproducible resource on earth, cheap substrate and do not compete with food chain such as FGB (Ferreira., 2010 ; Goh et al., 2010 ; Jurado et al., 2009).

Second generation bioethanol (SGB) is produce from lignocelluloses biomass, which is a non-edible sources, comprising mainly of cellulose, hemicellulose and lignin. Cellulose, which is a glucose polymer, hemicellulose, a mixture of polysaccharides composed mainly by galactose, rhamnose, mannose, xylose and arabinose, and lignin, which is a complex chemical compound most commonly derived from wood and function as support through strengthening of wood (xylem cells) in trees (Dias et al., 2009). These long chain polysaccharides can be hydrolyzed to produce a mixture of pentoses (C_5) and hexoses (C_6) which can be further converted into ethanol (Goh et al., 2010). Lignocelluloses are highly abundant and diverse in terms of availability. Cost of the feedstock is lower, and it can mitigate carbon emission and climate change caused by fossil fuel combustion, which emits excessive carbon monoxide and carbon dioxide (Yan and Lin, 2009).

Malaysia is one of the most important agricultural producer countries in the world. Therefore, it produces a lot of agriculture waste throughout a year. By converting the lignocelluloses biomass into ethanol, it will solve the disposal problem and reduce the cost of waste treatment (Goh et al. 2010). Examples of agriculture residues are such as empty fruit bunches (EFB) and annual or perennial plants such as kenaf and sorghum, which provide large amounts of biomass. In Malaysia, oil palm is one of the most important commercial crops. The explosive expansion of oil-palm plantation in Malaysia has generated large amount of agricultural waste, creating problems in disposal operations and environmental concerns. Studies have reported that Malaysia produced about 30 million tones annually of oil palm biomass in the recent years (Wan Rosli., 2004). Among various fibrous components of oil palm tree, EFB is the least to be studied including its conversion to bioethanol, but most studies focused on papermaking (Wan Rosli., 2007; Leh et al., 2008; Jimenez et al., 2009).

Besides agricultural waste, annual or perennial plants such as kenaf (Hibiscus cannabinus L.) has been identified by the Government and the National Tobacco Board (LTN) as a strategic crop and LTN has been actively identifying sustainable users of kenaf fibers. As well as raw material for fibrous products, kenaf also is a good candidate for bioethanol production because of its fast growth rate and large biomass production. Kenaf is a warm season annual herbaceous crop that belongs to Malvacea family and grows best in the tropics and subtropics where the mean daily temperatures during the growing season exceed 20°C. Kenaf can be physically separated into 2 parts – the long fiber bast and the short fiber woody core. Between the two fibers, kenaf core fiber, which is accounted to 60% of the kenaf stalk, has lower value for the production of fibrous product due to its relatively shorter fiber length and higher lignin content. Instead of fiber-based products production, kenaf core is a suitable raw material for bioethanol production.

Besides EFB and Kenaf core, Kapok (*Ceiba pentandra*) fibres are another source of fibers that are unicellular smooth, cylindrical and very lustrous. It is also reported that kapok fiber is six times lighter than cotton. It is buoyant, water repellent and slippery in nature. Moreover, it contains high cellulose properties due to its single cell fibers structure. Recently, kapok fibers were studied based on its chemical properties in order to use it as an oil absorbent, composite reinforcement and apparel application. (Mwaikambo et al., 2002; Hori et al., 2000).

Processing of lignocelluloses to bioethanol comprise the following main steps: hydrolysis of cellulose and hemicellulose, sugar fermentation, separation of lignin residue and ethanol recovery to meet specifications. However the task of hydrolyzing lignocelluloses to fermentable monosaccharide is still a major technical problem because the digestibility of cellulose is hindered by many physico-chemical structural and compositional factors. Therefore, pretreatment is an essential step for obtaining potentially fermentable sugars in hydrolysis step. Pre-treatment is required to separate lignin and hemicellulose structure from the cellulose, reduce cellulose crystallinity and increase the porosity of the substrate, thus improving cellulose hydrolysis (Alvira et al., 2010; Mosier et al., 2005). Pretreatment can be carried out in different ways such as mechanical pretreatment, biological pretreatment, chemical pretreatment such as alkali, acid, ozonolysis, organosolv, ionic liquids (ILs) and hydrothermal pretreatment such as steam explosion (autohydrolysis), liquid hot water, ammonia fiber explosion (AFEX), wet oxidation, microwave and ultrasound and thermo-chemical pretreatment such as gasification and pyrolysis (Alvira et al., 2010; Piccolo et al., 2009). Fig. 1 shows the schematic of pretreatment process.

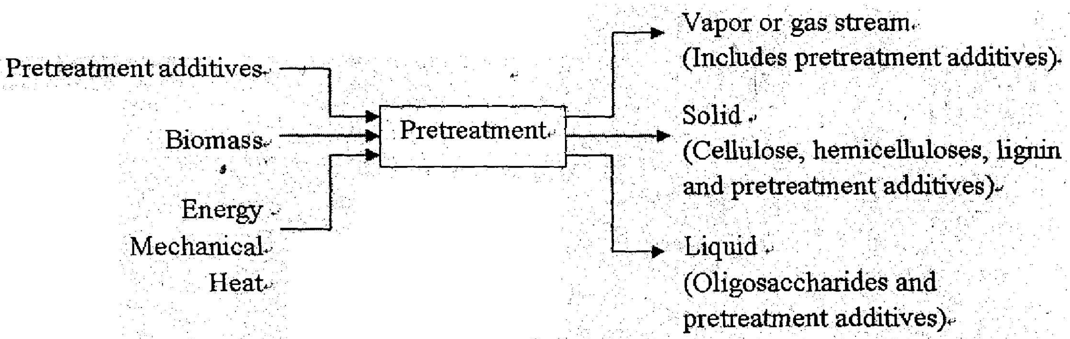


Fig. 1 Schematic of Pretreatment Process. (Mosier et al., 2005)

Generally, pretreatment of lignocelluloses biomass is usually fractionated by hydrolysis process. Chemical hydrolysis of biomass with dilute sulfuric acid has long been recognized as a critical step for removing hemicelluloses fraction from the lignocelluloses substrate to enhance the biological conversion of cellulosic biomass to ethanol. This method is cheap, safe and easy to handle, but it needs an elevated temperature to achieve high reaction rate. Unfortunately, the formation of inhibiting compound, such as furfural from degradation of pentose and 5-hydroxymethyl furfural (5-HMF) from degradation of hexose due to the elevated temperature can caused a reduction of total sugar recovery (Kuhad et al., 2010; Kahar et al., 2010; Teramoto et al., 2008). Some study demonstrates that the pretreatment such as acid hydrolysis followed by pulping process of *L. ca-mara* can provide high yield of cellulosic material and eventually be hydrolysed to hexose sugars for further fermentation into sugar. (Kuhad et al., 2010).

Among the different pretreatment method, chemical and thermo-chemical are currently the most effective and promising technologies for industrial application. Combination of different pretreatments has been also considered and might be interesting to obtain optimal fractionation of different components and reach very high yield (Alvira et al., 2010). A research group from the institute of Process Engineering, Chinese Academy of Sciences improved the ethanol yield from wheat straw with a pretreatment method of coupling steam explosion with alkaline peroxide to remove lignin from biomass. However the alkaline black liquor produced from this method can be very hazardous to the environment (Fang et al., 2010). Apart from that, the combination of dilute acid hydrolysis and wet explosion has been studied to increase the yield xylose and glucose in two separate processes that each can be optimized without negative effect on the sugar yield of other process (Sorensen et al., 2008). Besides, the pretreatment of wheat straw with 0.2% H_2SO_4 produced an easily digestible material, which resulted in high yields of glucose and xylose in enzymatic hydrolysis (Linde et al., 2008). Recently, acid pretreatment with additional pulping process has been investigated in order to produce a high yield of glucose in various applications such as bioethanol production and papermaking process (Mendes et al., 2009).

1.2 Objectives

This study embarks on the following objectives:

- To study the effect of water, acid and alkali pretreatments on chemical composition of various biomass.
- To investigate the potential of oxygen delignification treatment based on chemical composition of various biomasses.
- To study the feasibility and effectiveness of various pretreated lignocelluloses biomass for the production of ethanol.
- To study the effect of different pulping methods on acidic pretreated lignocelluloses biomass.
- To optimize processes' parameters through design of experiment.

2 METHODOLOGY

2.1 Description of Methodology

2.1.1 Raw Materials

Kapok, kenaf core and EFB fibre are used in this study. Kapok fibre is obtained by manually separated from the seed pod whereas the kenaf core fibre is ground in the length of 3-6 mm. The EFB fibrous strands are washed to remove oil and dirt. The air-dry EFB fibres are refined to size of about 2-4 cm before carrying any pretreatment or analysis.

Characterization of Raw Materials

The untreated raw materials are first ground and sieved (-20/+80 mesh). The extractive-free fibre dust is prepared according to TAPPI Standard Method T264 Preparation of Wood for Chemical Analysis with minor modification. After obtain the extractive-free fibres, the raw materials are continuous to others characterization procedure such as determination of holocellulose, cellulose, hemicellulose, lignin and carbohydrates content according to TAPPI Standard Methods. All analyses are performed in triplicate, and the results present as the mean ± standard deviation.

2.1.2 Pretreatments

Water, acid and alkali pretreatments are carried out in a 4-L stationary stainless steel digester (NAC Autoclave Co. Ltd., Japan) which is fitted with a microcomputer-controlled thermocouple. Upon completion of the pretreatment, the pretreated fibres are washed with tap water until neutral, and spin-dry before stored in the fridge for further use. The yields are determined based on oven-dry fibre weight.

- *Experimental Design*

Response surface methodology (RSM) is applied to obtain an estimation model of the total glucose yield (response variables) as a function of pretreatment parameters (i.e. temperature, time and chemical charge). The model is then analyzed based on its ANOVA and regression analysis with the assistance of statistical computer software, Design Expert® 6.0 (Stat-Ease, Inc. USA).

- *Characterization of Pretreated fibres*

The air-dry pretreated fibres are milled into small sizes, which can pass through a 2.0 mm pore-size sieve before chemical composition analysis. The pretreated fibres are quantified as extractive-free biomasses after the pretreatment. The chemical composition (holocellulose, cellulose, hemicellulose, lignin and carbohydrates contents) of pretreated fibres are tested according to TAPPI Standard Methods. All analyses are performed in triplicate, and the results present as the mean ± standard deviation.

2.1.3 Oxygen-alkali extraction

In turn, oxygen alkali extraction was conducted in a 1-L stainless high speed steel mixer, equipped with oxygen gas inlet, fabricated by local Malaysia company (Tai-Yi Machine (M) Sdn. Bhd). The oven-dried fibres (60g) were mixed with 1% v/v MgSO4 and 4% v/v NaOH, which were added based on the weight of oven-dried kenaf core fibre. The substrate mixture was then adjusted to 10% consistency by using distilled water. After placing the mixture in the mixing reactor, the cover was closed and fastened. The air valve was opened and the oxygen gas was introduced from a cylinder through the gas inlet to flush the air inside the reactor for 10 seconds. The air valve was then closed and the pressure inside the reactor was brought to 80 psi and kept at this level for the whole reaction time. Upon the completion of pretreatment (with and without oxygen-alkali extraction), all the pretreated kenaf core fibres were washed and spun-dried before stored in a fridge for further analysis. The solid yields were determined based on the weight of oven-dried fibre.

2.1.4 *Enzymatic hydrolysis*

Celluclast 1.5 L is used in this study. All enzymatic biomass hydrolyzes are carried out in 250 ml Erlenmeyer flasks. After the addition of the substrate, 0.05 M of citrate buffer solution (pH 4.8), and enzyme, the flasks are tightly sealed, and the mixtures are incubated in a shaking water bath (120 rpm) at a constant temperature (50 °C). After incubation, samples are boiled for 10 min to terminate the hydrolysis reaction. The mixtures are then centrifuged at 4000 x g for 10 min to remove unhydrolyzed residues. The supernatant is stored frozen (-4 °C) until analysis.

- *Enzyme (Cellulase) assay*

The enzyme uses in this study was Celluclast 1.5 L, which is a commercial cellulase supplied from Novozymes A/S Denmark. Its activity is assayed according to the Filter Paper Unit Assay (Ghose, 1987). The substrate uses in this assay is Whatman No.1 filter paper. 3,5-Dinitrosalysilic acid (DNS) is used to determine the reducing sugar that is released from the filter paper. The absolute amount of glucose releases is counted as reducing sugar. Anhydrous glucose is used as a standard for the reducing sugar measurement. The incubation conditions are 50 °C for 1 h. Cellulase activity is expressed as FPU/ml of the original enzyme solution.

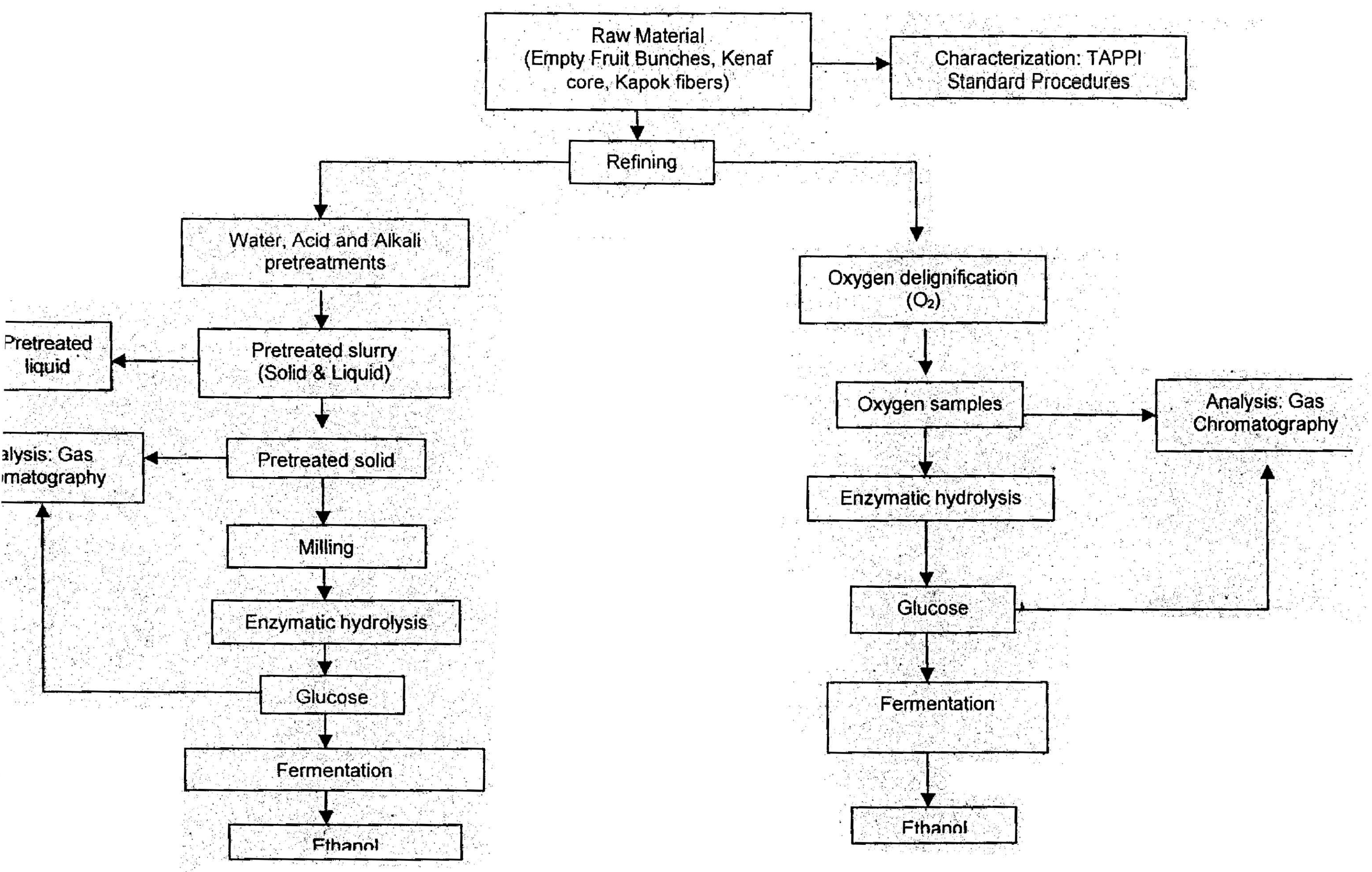
- *Reducing sugar determination*

The reducing sugar obtains from the filter paper is measured by using a dinitrosalysilic acid (DNS) assay (Miller, 1959). Sample is mixed with 3 ml DNS and boiled for exactly 5 min. The optical density is checked at 540 nm to measure the colour intensity. The reducing sugar as glucose is expressed in mg per ml. A standard curve of glucose is used for reducing sugar measurement. The amount reducing sugar contents in the supernatant of the enzymatic hydrolyzates is determined by a high-performance liquid chromatography (HPLC). The HPLC system (Agilent Tech. 385-ELSD) is equipped with a Hi-Plex Ca column (300 x 7.7 mm). Prior to injection, the samples (supernatant) are filtered by a 0.22 µm syringe filter. Next, 20 µl of the enzyme-free sample is loaded. The amounts of sugars produce are calculated according to calibration curves plotted with standard glucose.

2.1.5 *Fermentation*

The enzymatic hydrolyzate obtains from the fibre treated with optimum pretreatments condition is used to ferment into ethanol. Ethanol fermentation was conducted using 100 ml of enzymatic hydrolyzate in 250 ml Erlenmeyer flasks. A 10% (v/v) yeast inoculum was used. The flasks were fitted with two-holed rubber stoppers connected to rubber tubing. One of the rubber tubing was used for sample collection whereas another one allowed exhaustion of carbon dioxide. Fermentation was done at 30 °C and 150 rpm in the incubator shaker. Samples were withdrawn for analyses after 2, 4, 6, 8, 10, 12, 24 and 48 h, and centrifuged at 8000 x g for 10 min, for glucose (by HPLC) and ethanol (by GC) content analyses.

2.2 Flow chart of experiment



3 RESULTS AND DISCUSSION

3.1 PART I

3.1.1 *Preliminary study - Enzymatic hydrolysis of untreated kapok, EFB and kenaf core fibres*

3.1.1.1 Kapok fibre

The results of chemical composition and enzymatic hydrolysis of the untreated kapok fibre were shown in Table 1. In this study, its holocellulose content was 94.3%. Cellulose content was determined as a total amount of α- and ß-cellulose, which added up to 50.7%. Meanwhile, hemicellulose content was referred as γ-cellulose and amounted to 49.3%. Based on the results from gas chromatography, the glucose content of kapok fibre was approaching 60.0%, which made it a good substrate for ethanol production. On the other hand, the lignin content (Klason Lignin) in kapok fibre was merely 13.4%, which was relatively lower than other wood and non-wood biomass (Upendra and Shukla, 2010) (Table 1). Its comparatively high glucose and low lignin content exhibited greater potential on cellulosic ethanol production than other agricultural biomass (corn stover, wheat straw) and woody biomass (red pine, yellow poplar) (Zhu *et al.*, 2010). However, without any pretreatment, the kapok fibre released only a small amount of reducing sugar (0.04 g/L) after the enzymatic hydrolysis.

3.1.1.2 EFB fibre

According to Table 1, the cellulose content, as the summation of α- and ß-cellulose contents, of EFB fibre was 56.0%, while the hemicellulose content, which was quantified as γ-cellulose, was accounted to 44.0%. The results of carbohydrate analysis by gas chromatography showed that the glucose content of EFB fibre approached 60% sourced from cellulose and hemicellulose. Hence, EFB fibre was a promising substrate for the production of cellulosic ethanol. Another advantageous feature of EFB fibre was, in comparison to other biomass; it contains relatively lower Klason lignin content (<20%) (Upendra and Shukla, 2010). However, the result of enzymatic hydrolysis showed that the hydrolysability of the untreated EFB is very low; it released merely a small amount (0.66 g/L) of reducing sugar (glucose) and accounted to only 2.6% of glucose yield per weight of untreated fibre.

3.1.1.3 Kenaf core fibre

The cellulose and hemicellulose contents of untreated kenaf core fibre were 52.8% and 47.2%, respectively (Table 1). Since the glucose content of kenaf core fibre was exceeded 60%, it was a potential substrate for the cellulosic ethanol production. The Klason lignin content of kenaf core was approximately 20.0%, nevertheless; it was still verified lower than that of other biomass (Upendra and Shukla, 2010). Possibly, due to the higher Klason lignin content, the untreated kenaf core fibre was more difficult to be hydrolyzed by enzyme; it released only 0.1 g/L of glucose, which was corresponding to 0.4% of glucose yield per weight of untreated fibre.

Table 1 Chemical composition and enzymatic hydrolysis of untreated biomasses

	Untreated kapok fibre	Untreated EFB fibre	Untreated kenaf core fibre
Chemical composition of untreated fibres			
Holocellulose (%)	94.3±3.2	88.1±1.0	81.8±0.7
α-cellulose (%)	48.8±0.5	56.0±0.5	50.9±1.2
ß-cellulose (%)	1.9±0.6	<0.1±0.0	1.9±0.4
γ-cellulose (%)	49.3±1.1	44.0±0.2	47.2±0.9
Klason lignin (%)	13.4±0.4	15.1±0.1	20.3±0.2
Carbohydrates (GC analysis):			
Glucose (%)	59.8±0.2	56.2±0.7	66.4±0.6
Xylose (%)	33.8±0.0	38.9±0.7	26.6±0.6
Others sugar[a] (%)	6.5±0.2	4.9±0.1	7.92±0.41
Calculated glucose total content[b] (%)	56.4	47.8	54.3
Enzymatic hydrolysis of untreated fibres			
Theoretical glucose concentration[c] (g/L)	14.1	11.95	13.58
Glucose yield (HPLC analysis) (g/L)	0.04±0.0	0.66±0.1	0.1±0.02
Glucose yield based on:			
theoretical glucose concentration[d] (%)	0.3	5.5	0.7
pretreated substrate weight[e] (%)	0.2	2.6	0.4
untreated substrate weight[f] (%)	0.2	2.6	0.4

[a] Others sugar: arabinose, mannose, galactose

[b] Glucose content based on GC test (%) x Holocellulose content (%)

[c] Initial sample concentration (25g/L) x Calculated total glucose content (%) / 100

[d] = Theoretical glucose yield (%) = Glucose yield (g/L) / Theoretical glucose concentration (g/L) x 100

[e] = Enzymatic saccharification yield (%) = glucose yielded (g/L) / Initial sample concentration (25 g/L) x 100

[f] = Total glucose yield (%) = Enzymatic saccharification yield (%) x solid yield (%)

3.1.2 Optimization of various pretreatments on kapok, EFB and kenaf core fibres' for sugar production

To have better understanding on the effect of each pretreatment parameter on the sugar production, a detailed statistical analysis was required to carry out on each pretreatment. For this purpose, response surface methodology (RSM) was employed as the experimental design in the following study. Moreover, based on the statistical results, the optimum conditions for each pretreatment could also be determined as well. Instead of the enzymatic saccharification yield, the total glucose yield was taken as the response for the statistical study on the effect of pretreatments. The total glucose yield gives more meaning than the enzymatic saccharification yield for the pretreated fibre because it shows the percentage of total glucose that can be converted from a particular weight of original fibre directly.

3.1.2.1 Kapok fibre

The results for the response—total glucose yield based on each pretreatment condition were presented in Table 2. According to the statistical analysis provided by the experimental design software, a non-transformation quadratic model was adequate for all the pretreated biomasses. For water pretreatment, since all the factors were statistically significant, model reduction was not required. As shown in Table 2, this model was significant with a "probability > F" of less than 0.0001 and a regression coefficient (R^2) close to 1 as well as an insignificant of lack of fit (LOF). On the other hand, the quadratic response surface models for acid and alkaline pretreatments were reduced accordingly by eliminating some of the insignificant factors, with value of "Probability >F" more than 0.05. Based on the statistical analysis of ANOVA, both reduced models built were significant with values of "probability >F" less than 0.05, a regression (R^2) close to 1 and insignificant of LOF (Table 2). The response surfaces quadratic models for the response (total glucose yield) of the water, acid and alkaline pretreatments were shown by Equations 1 to 3.

Table 2 Results of total glucose yield based on each coded variables

Standard Order	Coded Variables			Responses
	T	t	c	Total glucose yield, %
Water pretreatment				
1	-2	-1	-	18.5
2	0	-1	-	36.0
3	1	-1	-	29.2
4	-2	0	-	21.2
5	0	0	-	29.5
6	1	0	-	22.1
7	-2	2	-	25.6
8	0	2	-	27.2
9	1	2	-	18.0
10	1	2	-	19.0
11	0	0	-	28.1
12	0	-1	-	34.0
13	-2	-1	-	19.0
Acid ptreatment				
1	-1	-1	0	23.0
2	-1	-1	1	14.6
3	0	-1	-1	32.8
4	1	-1	-1	26.9
5	-1	1	-1	16.7
6	0	1	0	29.9
7	0	1	1	27.1
8	1	1	0	22.0
9	1	1	1	17.2
10	0	1	0	27.3
11	-1	1	1	17.0
Alkaline pretreatment				
1	-1	-1	0	31.5
2	-1	-1	1	27.1
3	0	-1	-1	32.0
4	1	-1	-1	20.3
5	-1	1	-1	32.3
6	0	1	0	38.0
7	0	1	1	30.0
8	1	1	0	38.2
9	1	1	1	30.7
10	-1	-1	0	33.3
11	0	1	0	38.5

Table 3 Statistical analysis of reduced models and coefficient of total glucose yield of water, acid and alkaline pretreated kapok fibre

Water pretreatment			Acid pretreatment			Alkaline pretreatment		
Total glucose yield= Eq. 1			Total glucose yield= Eq. 2			Total glucose yield= Eq. 3		
Factor	Coeficient estimation, CE	Probability, Prob. >F	Factor	Coeficient estimation, CE	Probability, Prob. >F	Factor	Coeficient estimation, CE	Probability, Prob. >F
Intercept	28.91	-	Intercept	30.30	-	Intercept	32.36	-
T	0.47	0.4170	T	0.69	0.3723	T	-2.01	0.0219
t	-1.37	0.0351	t	-1.42	0.0480	t	4.73	0.0004
T^2	-8.63	< 0.0001	c	-2.45	0.0163	c	-5.96	0.0014
t^2	2.95	0.0244	T^2	-3.25	0.0332	T^2	-4.55	0.0069
Tt	-4.45	0.0002	c^2	-8.07	0.0013	Tt	7.37	0.0005
			ct	2.63	0.0277			
Model analysis			Model analysis			Model analysis		
Model prob. >F[a]	< 0.0001		Model prob. >F[a]	0.0027		Model prob. >F[a]	0.0016	
LOF prob. >F[b]	0.0928		LOF prob. >F[b]	0.7624		LOF prob. >F[b]	0.2234	
R^{2c}	0.9647		R^{2c}	0.9785		R^{2c}	0.9606	

T = Reaction temperature
t = Reaction time
c = Chemical concentration
[a] Value of "Prob. >F" less than 0.0500 indicates model is significant
[b] Non-significant lack of fit (LOF) (value of "Prob. >F" more than 0.0500) is good
[c] R^2 near 1 is good

Water pretreatment:

Total glucose yield

$$= +28.91 +0.47*T -1.37*t -8.63*T^2+2.95*t^2 -4.45*T*t \qquad \text{Eq. 1}$$

Acid pretreatment:

Total glucose yield

$$= +30.3 -2.45*c +0.69*T -1.42*t -3.25*c^2 -8.07*T^2 +2.63*c*t \qquad \text{Eq. 2}$$

Alkaline pretreatment:

Total glucose yield

$$= +32.36 -2.01T +4.73t -5.96c -4.55T^2 +7.377T*t \qquad \text{Eq. 3}$$

Based on the quadratic model built, the optimum water, acid and alkali pretreatments condition could be calculated by the computer software (Design Expert 6.0®). The condition with 170 °C for 45 min, 120 °C for 45 min in 1.0 % (v/v) H_2SO_4 solution and 120 °C for 60 min in 2.0 % (v/v) NaOH solution for water, acid and alkali pretreatments were selected as they achieved the highest total glucose yield. To verify the model, the selected optimum water, acid and alkali pretreatments condition were carried out experimentally in triplicate.

3.1.2.2 EFB fibre

Table 4 shows the results for the response—total glucose yield based on each pretreatments condition of EFB fibre. The statistical analysis results provided by the experimental design software indicated that a non-transformation quadratic model was obtained for all pretreated EFB fibres (Table 5). The quadratic response surface models for water, acid and alkaline pretreatments were reduced accordingly by eliminating some of the insignificant factors, which their value of "Probability >F" was more than 0.05. According to the ANOVA statistical analysis, the reduced models built of all samples were significant with the values of "probability >F" less than 0.05, a regression (R^2) closed to 1 and insignificant of LOF as well (Table 5). Therefore, the response surfaces quadratic models for the total glucose yield (the response) of the water, acid and alkaline pretreatments were shown by Equations 4 to 6.

Table 4 Results of total glucose yield based on each coded variables

Standard Order	Coded Variables			Responses
	T	t	c	Total glucose yield, %
Water pretreatment				
1	-2	-1	-	9.1
2	0	-1	-	40.0
3	1	-1	-	33.5
4	-2	0	-	22.2
5	0	0	-	40.9
6	1	0	-	33.8
7	-2	1	-	28.0
8	0	1	-	34.9
9	1	1	-	32.9
10	0	0	-	37.0
11	-2	-1	-	8.5
Acid ptreatment				
1	-1	-1	0	18.8
2	-1	-1	1	20.0
3	0	-1	-1	26.0
4	0	-1	1	23.0
5	1	-1	-1	10.2
6	1	-1	1	31.0
7	-1	1	-1	35.0
8	0	1	0	23.0
9	1	1	0	14.0
10	-1	1	1	22.0
11	0	1	0	33.4
Alkaline pretreatment				
1	-1	-1	0	28.0
2	-1	-1	1	32.4
3	0	-1	-1	26.8
4	0	-1	1	34.8
5	1	-1	-1	25.5
6	1	-1	1	22.6
7	-1	1	-1	17.4
8	0	1	0	28.8
9	1	1	0	24.7
10	-1	-1	1	31.9
11	0	1	0	27.9

Table 5 Statistical analysis of reduced models and coefficient of total glucose yield of water, acid and alkaline pretreated EFB fibre

Water pretreatment			Acid pretreatment			Alkaline pretreatment		
Total glucose yield= Eq. 5.9			Total glucose yield= Eq. 5.10			Total glucose yield= Eq. 5.11		
Factor	Coeficient estimation, CE	Probability, Prob. >F	Factor	Coeficient estimation, CE	Probability, Prob. >F	Factor	Coeficient estimation, CE	Probability, Prob. >F
Intercept	37.03	-	Intercept	31.35	-	Intercept	29.58	-
T	7.09	0.0013	c	1.57	0.3723	c	3.17	0.0026
t	3.13	0.0456	T	1.90	0.0480	T	-0.66	0.3452
T^2	-10.72	0.0041	T^2	-13.25	0.0163	T^2	-4.65	0.0043
Tt	-5.19	0.0066	cT	-4.60	0.0332	cT	-4.21	0.0015
Model analysis			Model analysis			Model analysis		
Model prob. >F^a	0.0005		Model prob. >F^a	0.0027		Model prob. >F^a	0.0012	
LOF prob. >F^b	0.2324		LOF prob. >F^b	0.3840		LOF prob. >F^b	0.0638	
R^{2c}	0.9510		R^{2c}	0.907		R^{2c}	0.9310	

T = Reaction temperature
t = Reaction time
c = Chemical charge
^{a}Value of "Prob. >F" less than 0.0500 indicates model is significant
^{b}Non-significant lack of fit (LOF) (value of "Prob. >F" more than 0.0500) is good
^{c}R^2 near 1 is good

Water pretreatment:
Total glucose yield

$$= +37.03 +7.09*T +3.13*t -10.72T^2 -5.19T*T*t \qquad \text{Eq. 4}$$

Acid pretreatment:
Total glucose yield

$$= +31.35 +1.57*c +1.9*T -13.25*T^2 -4.6*c*T \qquad \text{Eq. 5}$$

Alkaline pretreatment:
Total glucose yield

$$= +29.58 +3.17*c -0.66*T -4.65*T^2 -4.21*c*T \qquad \text{Eq. 6}$$

Based on the quadratic model built, the optimum water, acid and alkali pretreatments condition could be calculated by the computer software (Design Expert 6.0®). The condition with 170 °C for 45 min, 120 °C for 45 min in 2.0% (v/v) H2SO4 solution and 110 °C for 45 min in 3.0% (v/v) NaOH solution for water, acid and alkali pretreatments were selected as they achieved the highest total glucose yield. To verify the model, the selected optimum water, acid and alkali pretreatments condition were carried out experimentally in triplicate.

3.1.2.3 Kenaf core fibre

The results for the response—total glucose yield based on each pretreatments conditions of kenaf core fibre were as shown in Table 6. Moreover, the results presented in Table 7 were the variables and combined variables and their corresponding significant coefficient estimation (CE) for the response (total glucose yield) of water, acid and alkaline pretreatments. According to the statistical analysis results provided by the experimental design software, a non-transformation quadratic model was obtained for alkaline pretreated fibre. On the other hand, a transformation quadratic model was built for the water and acid pretreated fibres. Since the lack of fit of the non-transformed model for acid pretreatment was significant (values of "probability >F" less than 0.05), where actually an insignificant lack of fit is desired. Therefore, the squared root model was selected.

Table 6 The results of total glucose yield based on each coded variables

Standard Order	Coded Variables			Responses
	T	t	c	Total glucose yield, %
Water pretreatment				
1	-2	-1	-	1.1
2	0	-1	-	15.7
3	1	-1	-	3.0
4	-2	0	-	3.3
5	0	0	-	23.2
6	1	0	-	5.8
7	-2	1	-	4.5
8	0	1	-	19.3
9	1	1	-	7.3
10	0	0	-	22.1
11	0	1	-	18.9
Acid ptreatment				
1	-1	-1	-1	1.3
2	01	0	1	10.9
3	1	-1	-1	13.7
4	1	-1	1	12.6
5	-1	2	-1	6.0
6	-1	2	1	19.7
7	1	2	-1	8.0
8	1	2	1	10.2
9	-1	2	0	15.4
10	-1	0	0	6.1
11	1	2	0	13.2
12	1	0	0	12.0
13	1	0	0	13.0
Alkaline pretreatment				
1	-1	1	-1	13.9
2	-1	1	1	10.6
3	1	-1	-1	13.0
4	1	-1	1	16.4
5	0	1	0	12.6
6	0	1	0	12.4
7	-1	-1	0	8.7
8	1	1	0	12.7
9	0	-1	-1	11.3
10	0	-1	1	13.5
11	1	1	1	18.0

Table 7 Statistical analysis of reduced models and coefficient of total glucose yield of water, acid and alkaline pretreated kenaf core fibre

Water pretreatment			Acid pretreatment			Alkaline pretreatment		
Total glucose yield= (Eq. 6.9)^2			Total glucose yield= (Eq. 6.10)^2			Total glucose yield= Eq. 6.11		
Factor	Coeficient estimation, CE	Probability, Prob. >F	Factor	Coeficient estimation, CE	Probability, Prob. >F	Factor	Coeficient estimation, CE	Probability, Prob. >F
Intercept	4.8	-	Intercept	3.08	-	Intercept	11.24	-
T	0.31	0.0131	c	0.54	0.0004	c	0.4	0.2783
t	0.37	0.0043	T	0.28	0.0022	T	1.74	0.0032
T^2	-2.55	< 0.0001	t	0.18	0.0461	t	0.71	0.0491
t^2	-0.42	0.0537	c^2	-0.47	0.0129	c^2	2.12	0.0128
			t^2	0.46	0.0442	cT	1.7	0.0075
			cT	-0.49	0.0006			
			Tt	-0.47	0.0005			
Model analysis			Model analysis			Model analysis		
Model prob. >F^a	< 0.0001		Model prob. >F^a	0.0004		Model prob. >F^a	0.034	
LOF prob. >F^b	0.0537		LOF prob. >F^b	0.3967		LOF prob. >F^b	0.1132	
R^{2c}	0.9849		R^{2c}	0.9837		R^{2c}	0.9465	

T = Reaction temperature
t = Reaction time
c = Chemical charge
[a]Value of "Prob. >F" less than 0.0500 indicates model is significant
[b]Non-significant lack of fit (LOF) (value of "Prob. >F" more than 0.0500) is good
[c]R^2 near 1 is good

Furthermore, the quadratic response surface models for water, acid and alkaline pretreatments were reduced accordingly by removing some of the insignificant factors, which their value of "Probability >F" was more than 0.05. Based on the statistical analysis results of ANOVA, the reduced models built of all pretreated fibres, which were significant with the values of "probability >F" less than 0.05, a regression (R^2) closed to 1 as well as insignificant of LOF were shown in Table 7. Thus, the equation 7 to 9 illustrated the response surface quadratic models for the total glucose yield (the response) of the water, acid and alkaline pretreatments.

Water pretreatment:
Total glucose yield
$= (+22.62 + 1.2*T + 1.69*t - 16.59*T^2 - 2.79*t^2)^2$ Eq. 7
Acid pretreatment:
Total glucose yield
$= (+3.08 + 0.54*c + 0.28*T + 0.18*t - 0.47*c^2 + 0.46*t^2 - 0.49*c*T - 0.47*T*t)^2$ Eq. 8
Alkaline pretreatment:
Total glucose yield
$= +11.24 + 0.4*c + 1.74*T + 0.71*t + 2.12*c^2 + 1.7*c*T$ Eq. 9

Based on the quadratic model built, the optimum water, acid and alkali pretreatments condition were calculated by the computer software (Design Expert 6.0®). The optimum pretreatments condition was calculated and the condition with 170 °C for 45 min, 120 °C for 90 min in 2.0% (v/v) H_2SO_4 solution and 140 °C for 60 min in 3.0% (v/v) NaOH solution were selected. The selected water, acid and alkali pretreatment condition was employed experimentally in triplicate to further validate the model.

Table 8 Chemical composition and enzymatic hydrolysis of all pretreated fibres

	Water pretreated kapok fiber	Acid pretreated kapok fiber	Alkali pretreated kapok fiber	Water pretreated EFB fiber	Acid pretreated EFB fiber	Alkali pretreated EFB fiber	Water pretreated kenaf core fibre	Acid pretreated kenaf core fibre	Alkali pretreated kenaf core fibre
	170 °C, 45 min	120 °C, 1.0% v/v H_2SO_4, 45 min	120 °C, 2.0% v/v NaOH, 60 min	170 °C, 45 min	120 °C, 2.0% v/v H_2SO_4, 45 min	10 °C, 3% v/v NaOH, 45 min	170 °C, 45 min	120 °C, 2.0% v/v NaOH , 90 min	140 °C, 3% v/v NaOH, 60 min
Liquor pH	3.77	1.01	13.37	3.0	0.87	13.44	3.60	0.7	13.41
Solid Yield (%)	54.0±3.0^{A}	75.0±2.6^{C}	63.5±1.2^{B}	52.0±0.4^{A}	63.5±0.4^{B}	63.7±1.1^{B}	59.0±2.0^{C}	53.0±2.0^{B}	48.9±1.5^{A}
Chemical composition of pretreated kapok fiber									
Holocellulose (%)	82.3±2.1^{B}	81.7±0.1^{AB}	79.3±0.2^{A}	69.5±0.2^{A}	69.8±0.3^{A}	36.3±0.4^{B}	58.0±1.0^{B}	55.0±1.0^{A}	71.7±1.4^{C}
α-cellulose (%)	70.9±2.8^{A}	72.6±2.3^{A}	74.5±0.6^{A}	75.0±0.1^{B}	77.3±0.2^{C}	57.4±1.9^{A}	90.0±1.0^{C}	72.0±2.0^{A}	80.4±1.3^{B}
ß-cellulose (%)	19.1±0.1^{C}	10.5±0.2^{B}	1.9±0.4^{A}	19.0±0.3^{B}	22.7±0.6^{C}	3.0±0.7^{A}	9.0±1.0^{B}	28.0±2.0^{C}	2.3±0.1^{A}
γ-cellulose (%)	10.0±1.0^{A}	16.9±2.5^{B}	23.6±0.2^{C}	6.0±0.2^{B}	<0.1±0.0^{A}	39.6±1.1^{C}	1.0±0.1^{A}	<0.1±0.0^{A}	17.3±1.1^{B}
Klason Lignin (based on pretreated basis) (%)	19.1±0.8	16.7±0.1	9.3±1.0	18.6±0.2	28.6±0.1	3.7±0.7	46.0±1.0	52.0±1.0	18.0±0.1
(based on untreated basis) (%)	10.9±0.4^{B}	12.5±0.1^{C}	5.9±0.6^{A}	9.7±0.1^{B}	18.2±0.0^{C}	2.4±0.4^{A}	27.1±0.6^{B}	27.6±0.5^{B}	8.8±0.1^{A}
Acid Soluble Lignin (based on pretreated basis) (%)	1.0±0.0	1.6±0.1	2.1±0.3	1.5±0.3	1.3±0.2	1.0±0.1	0.6±0.0	0.6±0.1	0.8±0.1
(based on untreated basis) (%)	0.6±0.0	1.2±0.1	1.3±0.2	0.8±0.1	0.8±0.2	0.6±0.1	0.4±0.0	0.3±0.1	0.4±0.0
Carbohydrates:									
Glucose (%)	90.0±0.4	83.1±0.3	71.4±0.7	94.0±0.1	100.0±0.0	60.4±0.5	95.0±0.4	100±0.0	82.4±0.5
Xylose (%)	5.0±0.2	15.9±0.2	24.9±0.2	3.0±0.1	<0.1±0.0	39.6±0.5	2.0±0.0	<0.1±0.0	15.4±0.2
Others sugar [a] (%)	5.0±0.2	1.1±0.1	3.6±0.4	3.0±0.1	<0.1±0.0	<0.1±0.0	3.0±0.1	<0.1±0.0	2.3±0.2
Calculated Total Glucose Content [b] (%)	74.1±2.2	67.9±0.3	56.6±0.7	65.3±0.3	63.5±0.0	52.1±0.2	55.1±1.2	55.0±1.0	59.1±1.5
Enzymatic hydrolysis of pretreated kapok fiber									
Theoretical Glucose Concentration [c] (g/L)	18.5±0.6^{C}	17.0±0.1^{B}	14.2±0.1^{A}	16.3±0.1^{C}	15.9±0.1^{B}	13.0±0.2^{A}	13.8±0.3^{A}	13.8±0.2^{A}	14.8±0.4^{B}
Glucose yield (HPLC analysis) (g/L)	16.0±0.1^{B}	10.1±1.0^{A}	15.6±1.0^{B}	18.3±1.0^{B}	13.4±1.0^{A}	12.6±1.0^{A}	10.8±1.9^{B}	9.4±0.5^{A}	9.3±0.7^{A}
Glucose yield (HPLC analysis):									
Based on Theoretical glucose concentration [d] (%)	86.5±2.2^{B}	59.4±5.5^{A}	>99.9±0.0^{C}	>99.9±0.0^{B}	89.3±1.3^{A}	96.9±1.6^{B}	78.3±0.3^{C}	68.1±2.6^{B}	62.8±1.1^{A}
Based on Pretreated Substrate [e] (%)	64.0±0.4^{B}	40.4±4.0^{A}	62.4±4.0^{B}	73.2±4.0^{B}	53.5±4.1^{A}	50.4±1.6^{A}	43.2±0.8^{B}	37.7±1.9^{A}	37.2±0.4^{A}
Based on Untreated Substrate [f] (%)	**34.6±0.2^{A}**	**30.3±3.0^{A}**	**39.6±2.6^{B}**	**38.1±2.0^{B}**	**34.0±2.6^{A}**	**32.1±1.0^{A}**	**25.5±0.5^{B}**	**20.0±1.0^{A}**	**18.2±0.2^{A}**

[a] Others sugar: arabinose, mannose. galactose

[b, c, d, e, f] referred to Table 1

In each row values with the same capital letter are not significantly different, which analyzed by Duncan SPSS.

According to the theoretical glucose yield (the glucose yield calculated based on theoretical glucose concentration), one could say that more than 80% of the cellulose from most of the pretreated fibres (except pretreated kenaf core fibre) was hydrolyzed into glucose (Table 8). Therefore, pulping was not needed to apply in this study. Since there was only about 60-80% of the glucose (calculated based on theoretical glucose concentration) in the kenaf core fibres were hydrolyzed (Table 8), oxygen delignification treatment will be applied in all pretreated kenaf core fibre for further investigation (Kindly refer to Part II).

3.1.3 Fermentation of sugar enzymatic hydrolyzate into ethanol

3.1.3.1 Kapok fibre

Maximum ethanol concentration was achieved during 6-8 hours of fermentation for all enzymatic hydrolyzates (glucose) of pretreated kapok fibre. The decrease of glucose concentration was in tandem with the increase in the ethanol concentration during fermentation. The cell growth stopped (optical density value decreased and/or remained constant) when the glucose content was exhausted. It was also noted that the ethanol concentration reduced after about 10 hours of fermentation for all samples. This phenomenon indicated that Saccharomyces *cerevisiae* would firstly consume the glucose during high glucose concentration, and then started consuming the by-product (ethanol) when the glucose was depleted (Al-mhanna, 2010). According to Table 9, more than 95% of fermentation efficiency was achieved for all the pretreated fibre. This phenomenon revealed that the yeast strain used in this study would be able to tolerate various stress factors during fermentation such as nutrient deficiency, contamination, high glucose concentration, ethanol tolerance and its corresponding inhibition on the yeast cell growth and ethanol production (Patrascu *et al.*, 2009). Although the alkaline pretreated fibre showed the relatively lower fermentation efficiency (94.4%) than the other two fibres, it had attained the highest total ethanol yield (19.1%), due to its rather high total glucose yield (39.6%). Thus, the ethanol produced was directly related to the glucose content, for the range of glucose studied.

Table 9 Effects of different pretreatment (at optimum conditions, respectively) on total ethanol yield

Optimum Pretreatment condition	Total glucose yield (%)	Fermentation efficiency (%)	Total ethanol yield (%)
Kapok fibre			
Water pretreatment (170 °C, 45 min)	34.6±0.2^{A}	>99.9±0.0^{B}	17.6±0.1^{A}
Acid pretreatment (1.0% v/v H_2SO_4, 120 °C, 45 min)	30.3±3.0^{A}	>99.9±0.0^{B}	15.5±1.5^{A}
Alkaline pretreatment (2.0% v/v NaOH, 120 °C, 60 min)	39.6±2.6^{B}	94.4±1.0^{A}	19.1±1.2^{B}
EFB fibre			
Water pretreatment (170°C, 45 min)	38.1±2.0^{B}	95.4±1.0^{A}	18.5±1.0^{B}
Acid pretreatment (2.0% v/v H_2SO_4, 120°C, 60 min)	34.0±2.6^{A}	98.6±1.0^{A}	17.1±1.3^{A}
Alkaline pretreatment (3.0% v/v NaOH, 110°C, 45 min)	32.1±1.0^{A}	94.2±0.1^{A}	15.4±0.5^{A}
Kenaf core fibre			
Water pretreatment (170°C, 45 min)	25.5±0.5^{B}	97.9±1.0^{B}	12.7±0.2^{B}

Acid pretreatment (2.0% v/v H_2SO_4, 120°C, 90 min)	20.0±1.0^A	94.0±1.0^A	9.6±0.5^A
Alkaline pretreatment (3.0% v/v NaOH, 140°C, 60 min)	18.2±0.2^A	>99.9±0.0^C	9.3±0.1^A

3.1.3.2 *EFB fibre*

Maximum ethanol concentration was achieved during fermentation times of 10-12 hours for all enzymatic hydrolyzates (glucose) of pretreated EFB fibre. Similar to kapok fibre, the decrease of glucose concentration increased the ethanol concentration during fermentation. The cell growth stopped (optical density value decreased and/or remained constant) when the glucose content was exhausted. Moreover, it was observed that the ethanol concentration reduced after approximately 12 hours of fermentation for all samples. This phenomenon indicated that *Saccharomyces cerevisiae* would firstly consume the glucose during high glucose concentration, and subsequently started consuming the ethanol when the glucose content was getting low, as discussed earlier (Al-mhanna, 2010). According to Table 9, more than 98% of fermentation efficiency was attained for acid pretreated EFB fibre.

3.1.3.3 *Kenaf core fibre*

The maximum ethanol concentration was attained during 8-10 hours of fermentation for all enzymatic hydrolyzates (glucose) of pretreated kenaf core fibre. As stated earlier, the decrease of glucose concentration increased the ethanol concentration during fermentation. The cell growth stopped (optical density value decreased and/or remained constant) when the glucose entirely consumed. Moreover, it was found that the ethanol concentration reduced after about 10 hours fermentation of all samples. Thus, it was believed that the *Saccharomyces cerevisiae* would firstly consume the glucose when the glucose concentration was high, and then started consuming the ethanol when the glucose was run down (Al-mhanna, 2010), as discussed earlier. According to Table 9, without the nutrient supplementation, about 94% of fermentation efficiency was achieved for acid pretreated kenaf core fibre.

3.2 PART II

3.2.1 *Effects of various aqueous pretreatments on kenaf core fibres' chemical composition and enzymatic hydrolysability*

Raw kenaf core fibres consisted of 81.8% holocellulose, 52.8% cellulose (50.9% α-cellulose + 1.9% β-cellulose), 47.2% hemicellulose (γ-cellulose) and 20.3% Klason lignin. Moreover, carbohydrate analysis by gas chromatography indicated that holocellulose contained 66.4% glucose, 26.6% xylose and 7.9% other sugars (Tye et al., 2014). According to Table 10, all the pretreated kenaf core fibres showed an improvement on theirs cellulose and glucose contents, but a decrease on theirs holocellulose content. Generally, holocellulose is a combination of cellulose and hemicellulose fractions. It could be seen that the decrease of holocellulose content was mainly attributed by the removal of hemicellulose, which was demonstrated by the decrease of γ-cellulose content in the pretreatment samples (Table 10). Furthermore, the reduction of xylose content also denoted the effectiveness of pretreatments towards the hemicellulose removal as it is the main simple sugar presents in hemicelluloses (Tye et al., 2012). Among the pretreatments, acid pretreatment gave the best effect on elimination of hemicellulose. The hemicellulose (γ-cellulose) and xylose contents of acid pretreated fibre decreased effectively to 6.5% and 2.1%, respectively. In contrast, alkali pretreated fibre still retained very high hemicellulose content of 29.1% (or xylose content of 25.1%). This phenomenon was due to hemicellulose degraded more readily under acidic condition than alkali condition (Tye et al., 2014).

Table 10 Chemical analysis and enzymatic hydrolysis of water, acid and alkali pretreated kenaf core fibres

Pretreated sample	Water	Acid	Alkali
Chemical analysis			
Solid yield based on:			
pretreated sample weight (%)	62.3±4.1	62.6±2.4	59.8±2.8
untreated sample weight (%)	62.3±4.1	62.6±2.4	59.8±2.8
Holocellulose (%)	59.7±2.3	59.6±0.1	73.0±0.1
α-cellulose (%)	85.2±0.2	83.0±0.3	68.9±0.0
β-cellulose (%)	5.9±0.6	10.5±1.0	2.0±0.1
γ-cellulose (%)	8.8±0.4	6.5±0.6	29.1±0.1
Klason lignin based on:			
pretreated sample weight (%)	42.2±1.8	45.4±2.3	19.9±2.8
untreated sample weight (%)	26.3±1.1	28.4±1.5	11.9±1.7
Carbohydrates:			
Glucose	89.1±0.6	93.5±0.4	72.6±0.9
Xylose	6.0±0.1	2.1±0.0	25.1±0.1
Others sugar	4.9±0.5	4.4±0.4	2.3±0.7
Calculated glucose total content (%)	53.2±2.4	55.7±0.3	53.0±0.7
Enzymatic hydrolysis			
Theoretical glucose concentration (g/L)	13.3±0.6	13.9±0.1	13.3±0.2
Glucose yield (g/L)	6.0±0.1	2.5±0.4	5.5±0.1
Glucose yield based on:			
theoretical glucose concentration (%)	45.1±1.4	18.0±2.0	41.5±0.2
pretreated sample weight (%)	24.0±0.4	10.0±1.6	22.0±0.4
untreated sample weight (%)	**15.0±1.0**	**6.3±1.0**	**13.2±0.2**

Nevertheless, alkali pretreatment was more effectively on the removal of lignin. On the other hand, it was found that the lignin content of water and acid pretreated fibres were higher than that of the original one (20.3%). This phenomenon was due to the depolymerization and condensation of dissolved lignin during the pretreatment (Tye et al., 2014). Moreover, it was interesting to see that besides the degradation of lignin, alkali pretreatment was also capable of removing more than 35% of hemicellulose (γ-cellulose) from kenaf core fibre. Besides, it was noted that the cellulose content of water and acid pretreated fibres were greater than and equal to the glucose content, respectively (Table 10). Hence, it was believed that the glucose content of these pretreated fibres was wholly contributed by the cellulose and none from hemicellulose. On the other hand, the amount of glucose content of alkali pretreated fibre was larger than that of the cellulose content (Table 10). This indicated that the glucose content was not only contributed by cellulose but also hemicellulose. Or in other words, the remaining hemicellulose content in the alkali pretreated fibre contained a small part of the glucose.

Based on the results presented in Table 10, alkali pretreatment exhibited the highest ability to remove lignin (41.4%) whereas both water and acid pretreatments revealed the highest ability to eliminate hemicellulose (>80%). Nevertheless, water pretreated fibre attained the highest total glucose yield, followed by alkali and acid pretreated fibres. Although alkali pretreated fibre showed about 40% of lignin and hemicellulose contents removal, its fibre hydrolysability was relatively lower than the water pretreated one, in which the glucose hydrolyze from the former and latter were 41.5% and 45.1%, respectively. Hence, this suggested that the removal of hemicellulose was more crucial than the removal of lignin in improving the kenaf core fibre hydrolysability. Besides, it was noted that acid pretreated fibre experienced better hemicellulose removal than the water pretreated fibre did. However, the total glucose yield of latter case was achieved about two folds of the former case (Table 10). This result was probably attributed by the high remaining of residual lignin in the acid pretreated fibre, which could absorb the enzyme and consequently, decreases the enzyme activity during hydrolysis.

3.2.2 *Effects of various aqueous pretreatments followed with oxygen-alkali extraction on kenaf core fibres' chemical composition and enzymatic hydrolysability*

With the simple aqueous pretreatments, the enzymatic hydrolysability of kenaf core fibres was enhanced. However, these pretreated fibres achieved only less than 50% of theoretical glucose yields (Table 10). Therefore, oxygen-alkali extraction was introduced in this study due to its capable of removing lignin from biomass in an environmentally friendly way. It could be observed that although the effect of alkali pretreatment was analogue to the oxygen-alkali extraction, in which both methods were capable of removing lignin, the hemicellulose content of the latter was about 45% higher than that of the former (Table 10 and 11). This denoted that the oxygen-alkali extracted fibre retained most of the hemicellulose and thus contributed more glucose content in comparison to the alkali pretreated fibre did. Nevertheless, the theoretical glucose yield as well as the total glucose yield of oxygen-alkali extracted fibre was still low (Table 11). Hence, it was believed that these pretreatment and extraction conditions were insufficient to improve the enzymatic hydrolysability of fibre.

As the simple aqueous pretreatments and oxygen-alkali extraction solely could not enhance the kenaf core fibre's enzymatic hydrolysability up to the satisfactory saccharification yield (>50%), the combination of the two treatments was undertaken for further investigation. As shown by Table 12, it was obvious that with the two-stage pretreatments (simple aqueous pretreatments followed by oxygen-alkali extraction) the glucose yield (based on HPLC analysis) of all the pretreated fibres were improved markedly. Moreover, it was surprisingly to observe that all the pretreated fibres could recover in more than 75% of solid yield and 80% of theoretical glucose yield. Hence, the total glucose yields of these two-stage pretreated fibres were about double the yield of the pretreated fibres without oxygen-alkali extraction.

Based on Table 12, combination pretreatments did not severely degrade the cellulose, in which only less than 2.5% of cellulose lost was observed. Nevertheless, the reduction of solid yield was mainly due to the removal of hemicellulose and/or lignin from the fibres. In comparison to water and acid pretreatment, both water and acid pretreatment followed with oxygen-alkali extraction process were capable of removing lignin in a great extent, which further removed about 25% and 32% of lignin, respectively. In contrast, the lignin content of alkali-oxygen pretreated fibre still remained the same as the content in alkali pretreated fibre. This phenomenon indicated that oxygen-alkali extraction process was more efficient on the removal of lignin for fibres that had been treated with a low pH medium. The effect of water pretreatment was analogues to that of acid pretreatment; however, water-oxygen pretreatment exhibited the highest ability in removing hemicellulose as well as xylose, in comparison to acid-oxygen pretreatment. Apart from that, alkali-oxygen pretreated fibre retained slightly higher contents of both hemicellulose and other sugars than those in alkali pretreated fibre (Table 12). This finding revealed that alkali-oxygen pretreatment would not further degrade the carbohydrates.

Among the two-stage pretreated fibres, acid-oxygen pretreated fibre exhibited the highest total glucose yield followed by alkali-oxygen and water-oxygen pretreated fibres. It could be seen that although both water-oxygen and acid-oxygen pretreated fibres presented the low amount of hemicellulose content, the total glucose yield of the former was relatively lower about 6.3% than that of the latter. The chemical composition analysis (Table 12) showed that water-oxygen pretreated fibre experienced more cellulose degradation (2.4%) than the acid-oxygen pretreated fibre (1.7%) did. Its relatively high cellulose degradation caused the calculated values of both the total glucose content (59.1%) and theoretical glucose concentration (14.8 g/L) low. These results indicated that the enzymatic saccharification yield of the water-oxygen pretreated fibre (49.6%) would not be higher than 59.1%. Hence, the total glucose yield calculated from the enzymatic saccharification yield multiplied by solid yield was only 24.9%. Furthermore since the substantially high lignin content (28.4%) remained in the acid pretreated fibre could decrease the enzymatic saccharification yield as well as total glucose yield; it was believed that acid-oxygen pretreated fibre attained the remarkably high total glucose yield was attributed by its rather low lignin content (Table 12).

Table 11 Chemical analysis and enzymatic hydrolysis of oxygen-alkali extracted kenaf core fibre

Extracted sample	Oxygen-alkali
Chemical analysis	
Solid yield based on:	
pretreated sample weight (%)	68.0±3.0
untreated sample weight (%)	68.0±3.0
Holocellulose (%)	79.3±1.4
α-cellulose (%)	55.6±1.0
β-cellulose (%)	1.4±0.4
Ƴ-cellulose (%)	43.0±0.6
Klason lignin based on:	
pretreated sample weight (%)	22.8±2.2
untreated sample weight (%)	15.5±1.5
Carbohydrates (GC analysis):	
Glucose	65.6±0.9
Xylose	27.8±0.1
Others sugar	6.6±0.7
Calculated glucose total content (%)	52.0±0.7
Enzymatic hydrolysis	
Theoretical glucose concentration (g/L)	13.0±0.2
Glucose yield (HPLC analysis) (g/L)	5.5±0.1
Glucose yield based on:	
theoretical glucose concentration (%)	42.3±0.2
pretreated sample weight (%)	22.0±0.4
untreated sample weight (%)	15.0±0.2

Unlike water and acid pretreatment, alkali pretreatment followed with oxygen-alkali extraction process did not dramatically change the chemical composition of the fibre. However, the total glucose yield of alkali-oxygen pretreated fibre was improved remarkably (Table 12). It was believed that besides the removal of amorphous fractions (lignin and/or hemicellulose), there were some other changes, such as the physical structure of fibre, might affect the enzymatic hydrolysability of the fibre. Therefore, further research on this study is needed.

Table 12 Chemical analysis and enzymatic hydrolysis of various two-stage pretreated kenaf core fibres

Pretreated sample	Water-Oxygen	Acid-Oxygen	Alkali-Oxygen
Chemical analysis			
Solid yield based on:			
pretreated sample weight (%)	80.7±4.0	78.0±2.0	90.4±3.0
untreated sample weight (%)	50.3±3.1	48.8±1.4	54.1±1.8
Holocellulose (%)	66.1±1.8	72.4±0.8	82.6±2.0
α-cellulose (%)	88.4±1.0	83.4±0.0	67.0±2.0
β-cellulose (%)	6.3±0.8	10.5±0.8	2.0±0.0
Ƴ-cellulose (%)	5.3±0.2	6.1±0.8	32.0±0.0
Klason lignin based on			
pretreated sample weight (%)	39.0±2.8	39.5±2.8	21.7±2.2
untreated sample weight (%)	19.6±1.4	19.3±1.3	11.7±1.2
Carbohydrates:			
Glucose	89.4±0.4	93.2±0.2	71.6±0.6
Xylose	4.2±0.2	2.6±0.2	24.0±0.2
Others sugar (1)	3.1±0.2	4.2±0.0	4.4±0.4

Calculated glucose total content (%)	59.1±0.1	67.5±0.5	64.7±0.7
Enzymatic hydrolysis			
Theoretical glucose concentration (g/L)	14.8±0.1	16.9±0.1	16.2±0.2
Glucose yield (g/L)	12.4±0.4	16.0±0.6	14.0±0.5
Glucose yield based on:			
theoretical glucose concentration (%)	83.8±0.8	90.7±0.7	86.4±0.4
pretreated sample weight (%)	49.6±0.6	64.0±0.4	56.0±0.5
untreated sample weight (%)	**24.9±0.9**	**31.2±0.4**	**30.3±0.3**

4 CONCLUSION

4.1 Part I

- The removal of the entire recalcitrant polymers (lignin and hemicellulose) from biomass by pretreatment was not necessary to maximize (>95%) the conversion of cellulose into reducing sugar by enzymatic hydrolysis. Thus, only simple pretreatments without fully removing of lignin and /or hemicellulose could improve the fibre enzymatic hydrolysability to approach full cellulose-glucose conversion.
- Pretreatments improved the enzymatic hydrolysability of biomass through the removal of parts of the recalcitrant polymers. However, the removal of lignin was not the compulsory requirement to improve the enzymatic hydrolysability of biomass while the removal of hemicellulose might be the major criterion to do so. Thus the main factor probably highly related to the linkage or arrangement among lignin-hemicellulose-cellulose.
- Based on the results of Kenaf core fibre, the removal of recalcitrant polymers by simple pretreatments were not sufficient to maximize the enzymatic hydrolysability of the fibre. This indicated that there are other factors may interfere the enzymatic hydrolysis for certain biomass, which could not be concluded based on the results within the scope of this study.
- Different biomasses required different pretreatments to improve their enzymatic hydrolysability. The optimum pretreatment condition for kapok fibre was the alkaline pretreatment with condition of 120 °C for 60 min in 2.0% NaOH solution. Whilst the optimum pretreatment conditions for EFB and kenaf core fibres were the same, which were the water pretreatment with condition of 170 °C for 45 min.
- Sugar hydrolyzates from impure pretreated biomasses could be fermented into ethanol with high fermentation efficiency.

4.2 Part II

- The enzymatic hydrolysability of the kenaf core fibres was enhanced by adopting simple aqueous pretreatments; however it did not up to satisfactory saccharification yield (only less than 50%).
- The combination of aqueous treatment with oxygen-alkali extraction (two-stage pretreatment) was able to further improve the enzymatic hydrolysability of the fibre and increased the theoretical glucose yield up to more than 80%.
- Although the total glucose yields of all the two-step pretreated fibres were merely slightly different, acid-oxygen pretreatment was the most favourable method on enhancing the enzymatic hydrolysability of kenaf core fibre as its total glucose yield improved about five folds in relative to the acid pretreated one.

5 RECOMMENDATIONS

- For kenaf core fibre, instead of simple pretreatment, others advance pretreatment methods such as ionic liquid pretreatment are suggested for bioethanol production.

- Using the other pretreatment methods such as phsico-chemical pretreatment and combination of simple pretreatmet methods are recommended.

- Identify some other factors that may influence the enzymatic hydrolysability of the fibre such as chemical linkages and bonding characteristics of the untreated and pretreated fibres.

- Produce bioethanol by using the pretreatment liquor.

6 ACKNOWLEDGEMENTS

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Saya telah memeriksa dan menyemak setiap alatan dan didapati :

☒ Lengkap
☐ Rosak
☐ Hilang : Nyatakan...
☐ Lain-lain : Nyatakan ....

Diperakukan Oleh :

29/09/2016

....
Tandatangan
Pegawai Aset PTJ

Nama : KU ABDULLAH B. CHE KU ALAM
PEGAWAI PENYELIDIK
PUSAT PENGAJIAN TEKNOLOGI INDUSTRI
UNIVERSITI SAINS MALAYSIA
11800 USM [illegible] PINANG

Tarikh : 26/09/2016..........

***Nota :** Sesalinan borang yang telah lengkap perlulah dikemukakan kepada Unit Pengurusan Harta, Jabatan Bendahari dan Pejabat RCMO untuk tujuan rekod.

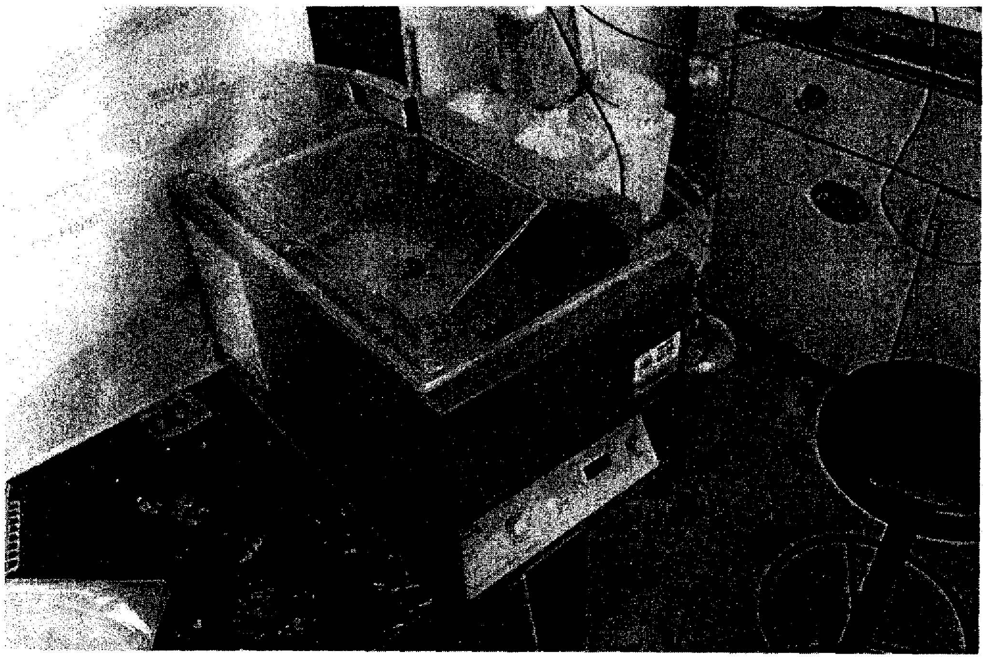
PUSAT PENGAJIAN TEKNOLOGI INDUSTRI

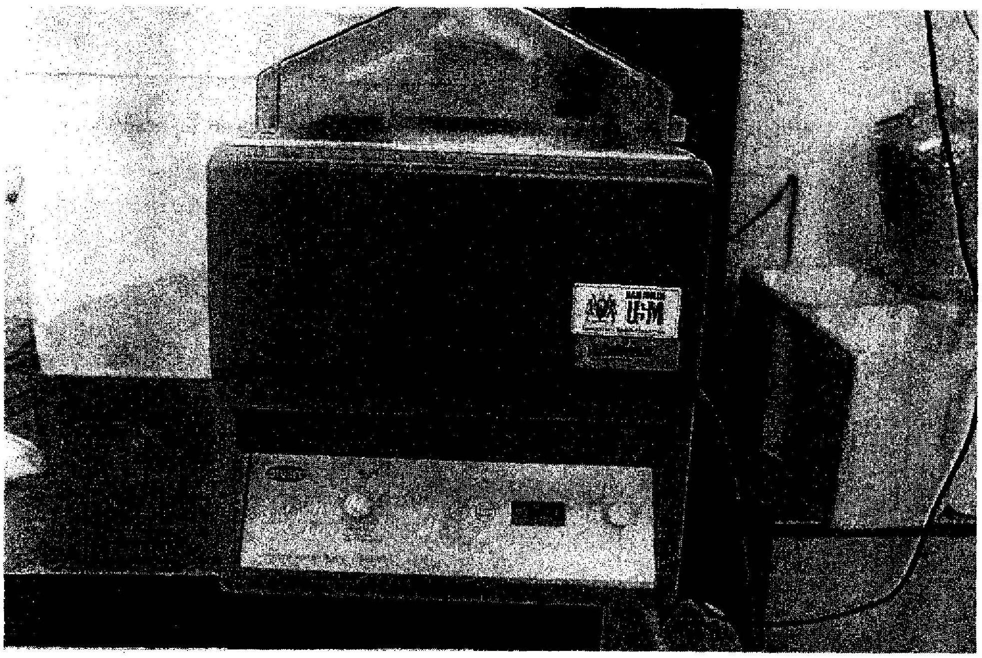
PENYERAHAN ASET

DR LEH CHEU PENG

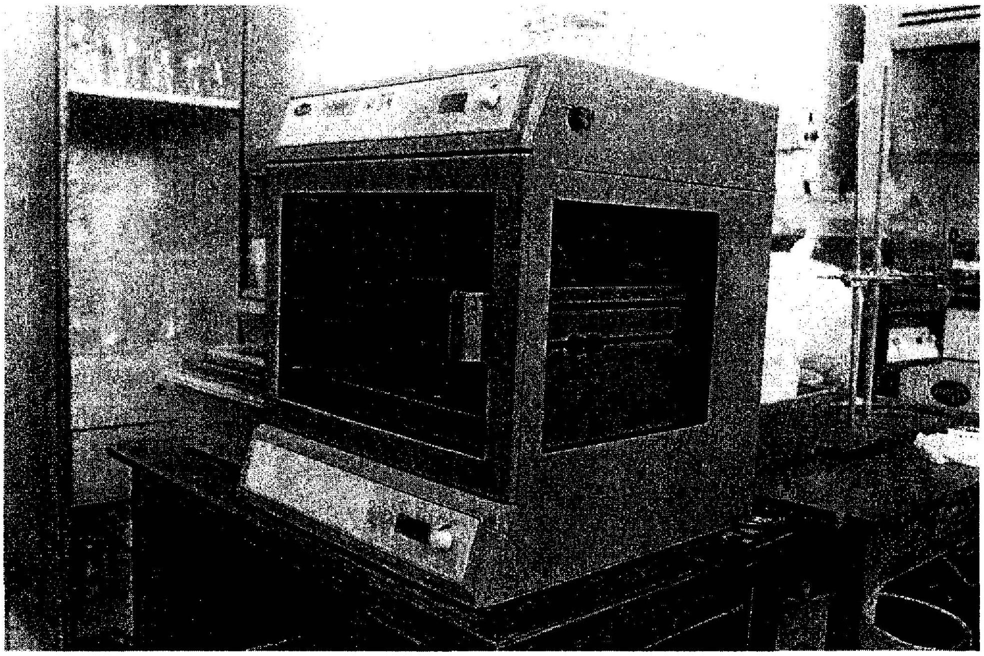
29 SEPT 2016

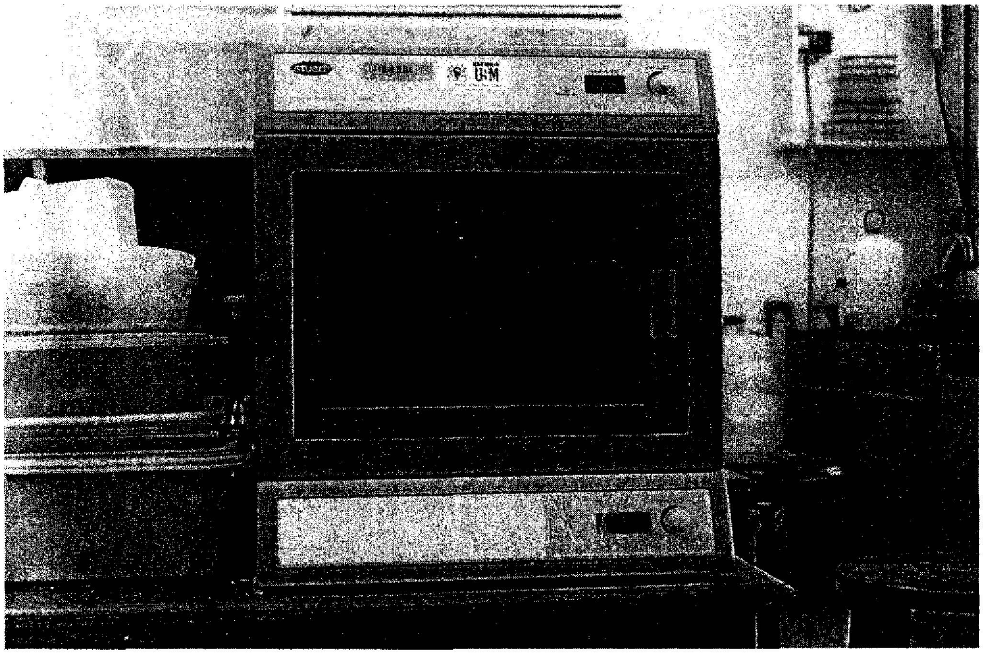
ALAT	SHAKER WATER BATH
LOKASI	229
SERIAL NUMBER	R000100588
NO HARTA	1AK00048373PTEKIND 2012
PENSYARAH BERTANGGUNGJAWAB	Dr. LEH CHEU PENG
BAHAGIAN / DIVISION	BPC

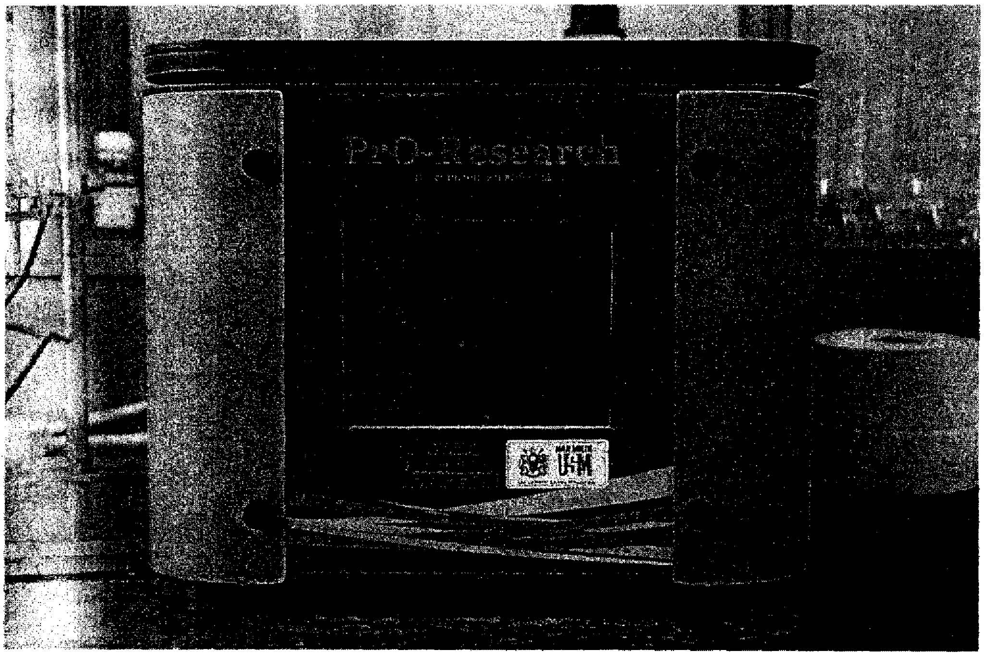
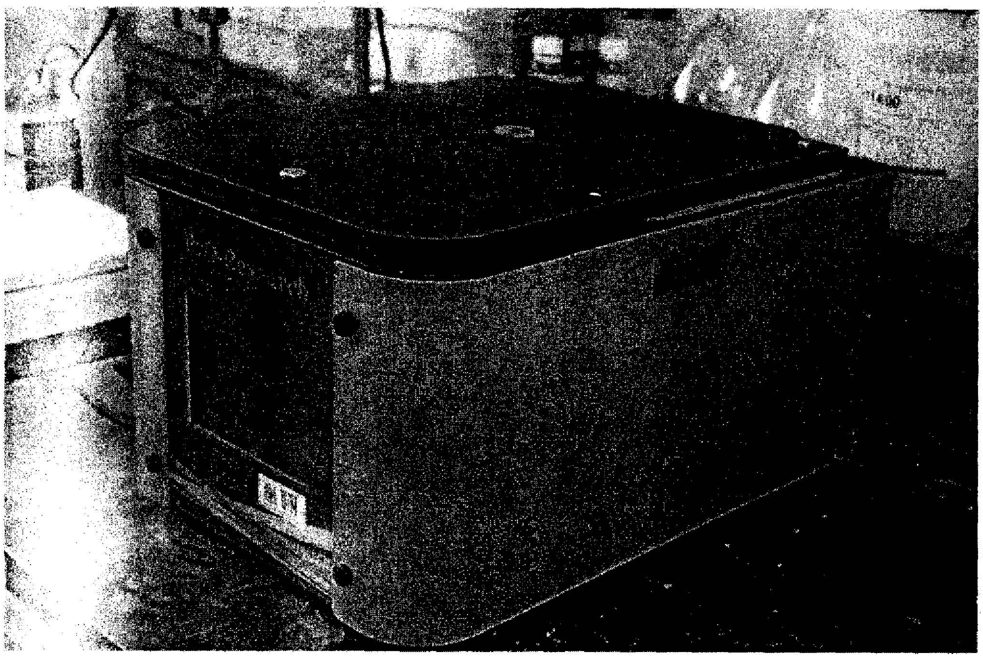




ALAT	SHAKER INCUBATOR
LOKASI	229
SERIAL NUMBER	R000100961
NO HARTA	1AK00048252PTEKIND 2012
PENSYARAH BERTANGGUNGJAWAB	Dr. LEH CHEU PENG
BAHAGIAN / DIVISION	BPC





ALAT	CENTRIFUGE MACHINE
LOKASI	229
SERIAL NUMBER	216379
NO HARTA	1AK00051731PTEKIND 2015
PENSYARAH BERTANGGUNGJAWAB	Dr. LEH CHEU PENG
BAHAGIAN / DIVISION	BPC
	
	

ALAT	REFRIGERATOR
LOKASI	229
SERIAL NUMBER	108KRJU2W868
NO HARTA	-
PENSYARAH BERTANGGUNGJAWAB	Dr. LEH CHEU PENG
BAHAGIAN / DIVISION	BPC