COMBINATION OF LOW PRESSURE STEAM HEATING AND DILUTE ACID PRETREATMENT OF PALM BIOMASS FOR FERMENTABLE SUGAR PRODUCTION

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Dedicated to my beloved Ibu, Ayah, Families
And to my supervisors, Dr Umi Aisah , Dr Zainul and friends for
endless help and support

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ABSTRACT

Bioethanol derived from empty fruit bunch (EFB) biomass can be potentially used to supplement increasing demand of fossil fuels. Carbohydrate fraction of EFB is an excellent property to be exploited as fermentable sugars that can be converted into bioethanol. In order to produce fermentable sugars from EFB, pretreatment process is required to break complex structure. This process is a challenging process due to incalcitrant structure of lignocellulosic material. With this regards, extensive researches have been done to improve efficiency of pretreatment method for maximum fermentable sugars production. In this study, the attempt to employ a combination of low pressure steam heating (LPSH) and dilute acid pre-treatment for fermentable sugar production was investigated. A dilute acid pretreatment was chosen after the preliminary study was done in comparison with other two different pretreatments, which were lime and ammonia method. In this study, a two-stage-acid hydrolysis was used after the pretreatment step. The hydrolysate was then fermented by Baker's yeast to convert the released glucose into bioethanol. The saccharification of sugar yields was evaluated using glucose kit Boehringer Mannheim/R-Biopharm and Megazyme xylose kit whereas morphological changes were determined using scanning electron microscopy and crystallinity was determined using X-ray diffraction . The low pressure pretreatment method was carried out in a pressure cooker. The first attempt of utilization of LPSH method at a pressure of 5 psi followed by dilute acid pre-treatment using 4% (v/v) sulphuric acid has shown an increment from 43.1 to 62.1 % (w/w) fermentable sugars compared to dilute acid alone. Further study was done using LPSH at different residence time from 5 to 45 minutes with combination, at different concentration of 1 to 4% (v/v). From this study, the maximum fermentable sugars obtained was 78.6% (w/w) by increasing the LPSH condition to 10 psi, for 30 minutes with combination of 3% (v/v) of dilute sulfuric acid pretreatment with an increment of 45% of glucose as compared to single pretreatment. The combination strategy of pre-treatment was proven to be an effective approach to enhance sugar increment. From the sugars obtained, 8.87mg/mL of ethanol was produced from the fermentation process which proves that the sugar obtained is fermentable.

ABSTRAK

Bioetanol yang dihasilkan daripada sisa biojisim tandan kosong kelapa sawit (EFB) berpotensi untuk digunakan sebagai tambahan kepada peningkatan permintaan bahan api fosil. Pecahan karbohidrat EFB mempunyai ciri yang baik untuk dieksploitasi sebagai gula-mudah tapai dan boleh ditukarkan kepada bioetanol. Dalam usaha untuk menghasilkan gula-mudah tapai dari EFB, proses prarawatan diperlukan untuk memecahkan struktur yang kompleks. Proses ini adalah satu proses yang mencabar kerana struktur tegar bahan lignoselulosa. Oleh yang demikian, kajian yang menyeluruh telah dilakukan bagi meningkatkan kecekapan kaedah pra-rawatan untuk menghasilkan gula-mudah tapai secara maksimum. Di dalam kajian ini, percubaan untuk menggunakan gabungan prarawatan pemanasan stim tekanan rendah (LPSH) dan asid cair untuk menghasilkan gula-mudah tapai secara maksimum telah dilakukan. Rawatan asid cair dipilih setelah kajian awal dilakukan untuk membandingkan prestasi prarawatan dengan dua prarawatan yang berbeza, iaitu kapur dan ammonia. Dalam kajian ini, asid hidrolisis dua peringkat telah digunakan selepas proses prarawatan. Hidrolisat yang diperoleh ditapaikan dengan menggunakan yis Baker untuk menukarkan gula kepada bioetanol. Gula yang terhasil daripada pensakaridaan telah diuji menggunakan kit glukosa *Boehringer Mannheim / R-Biopharm* dan xilosa *Megazyme* manakala perubahan morfologi telah dikaji dengan menggunakan mikroskopi pengimbasan elektron dan penghabluran ditentukan menggunakan pembelauan sinar-X. Kaedah prarawatan menggunakan tekanan rendah telah dilakukan di dalam periuk tekanan. Percubaan pertama menggunakan kaedah LPSH pada tekanan 5 psi diikuti dengan rawatan asid cair menggunakan 4% asid sulfurik telah menunjukkan peningkatan daripada 43.1 kepada 62.1 % (w / w) gula-mudah tapai berbanding menggunakan kaedah rawatan asid cair sahaja. Kajian lanjut telah dilakukan menggunakan tempoh rawatan LPSH yang berbeza iaitu 5-45 minit bersama gabungan kepekatan sulfurik asid dari 1 hingga 4% (v/v). Daripada kajian ini, didapati bahawa gula maksimum yang diperoleh ialah 78.6% (w/w) dengan menaikkan LPSH kepada 10 psi, tempoh rawatan selama 30 minit bersama gabungan 3% (v/v) asid sulfurik cair dengan peningkatan sebanyak 45% glukosa jika dibandingkan dengan menggunakan satu kaedah prarawatan sahaja. Strategi penggabungan prarawatan telah terbukti berkesan untuk meningkatkan penghasilan gula. Dari gula yang diperoleh, 8.87mg/mL etanol berjaya dihasilkan melalui proses penapaian dan berjaya membuktikan gula yang terhasil boleh ditapaikan.

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LIST OF ABBREVIATIONS

APEX	-	Ammonia Fiber/ Freeze Explosion
ASL	-	Acid Soluble Lignin
BMIMCI	-	1-N-Butyl-3- Methyllimidazolium Chloride
$Ca(OH)_2$	-	Calcium Hydroxide
CrI	-	Crystallinity Index
DAP	-	Dilute Acid Pre-treatment
DOE	-	US Department of Energy
DP	-	Degree of Polymerization
EDA	-	Electron Donor Accepter
EFB	-	Empty Fruit Bunch
FPU	-	Filter Paper Unit
GHG	-	Greenhouse Gases
H_2SO_4	-	Sulphuric Acid
HCI	-	Hydrochloride acid
HMF	-	Hydroxymethylfurfural
HPLC	-	High Performance Liquid Chromatography
ILs	-	Ionic Liquid
KOH	-	Potassium Hydroxide

LAPs	-	Laboratory Analytical Procedures
LHW	-	Liquid Hot Water Treatment
LPSH	-	Low Pressure Steam Heating
MPOB	-	Malaysian Palm Oil Board
Mtoe	-	Million tonne of oil equivalent
NaOH	-	Sodium Hydroxide
NH3	-	Ammonia
NH4CI	-	Ammonium Chloride
NMMO	-	N-Methylmorpholine-N-oxide Monohydrate
NREL	-	National Renewable Energy Laboratory
rpm	-	Revolutions per minutes
SE	-	Steam Explosion
SEDA	-	Sustainable Energy Development Authority Malaysia
SEM	-	Scanning Electron Microscopy
SO_2	-	Sulphur dioxide
SSF	-	Simultaneous Saccharification and Fermentation
UV-Vis	-	UV-Vis
XRD	-	X-ray diffraction

LIST OF SYMBOLS

%	-	Percentage
ε	-	Extinction coefficient
α	-	Alpha
Δ	-	Delta
°C	-	Degree Celcius
wt%	-	Weigh per weight
mg/ml	-	Milligram per mililiter
ml	-	Mililiter
cm	-	Centimeter
mmol	-	Milimole
v/v	-	Volume per volume
w/v	-	Weigh per volume
kV	-	Kilo volt
K	-	Kelvin
N	-	Normality
Mpa	-	Megapascal
psi	-	Pound per square inch
g/l	-	Gram per liter
μl/ml	-	Microliter per mililiter

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Progressing towards the 21st century, the world is currently facing an accelerating consumption of energy due to rapid population and economic growth especially China and India. Currently, the world population reaching 7 billion people and it will rise in the near future (Haub and Kaneda, 2013). Malaysia in particularly, the energy consumptions were expected to reach a rapid increase of 98.2 million tonne of oil equivalent (Mtoe) by 2030 (Shuit *et al*., 2009). Figure 1.1 illustrated the trend of energy demand in Malaysia from 1980 to 2030.

As most energy sources are derived mainly from fossil fuel, there is a concern where a dependency on oil will lead to energy shortage as the oil reserved is depleting. In addition, burning fossil fuels contributed to environmental problems such as global climate change through greenhouse emission (GHE). With reference to these problems, an alternative energy especially renewable energy must be explored to decrease a dependency on fossil fuel. With this regards, Malaysian government has implemented policy such as National Renewable Energy Policy 2010, (SEDA, 2014) to enhance the usage of renewable energy as biofuel.

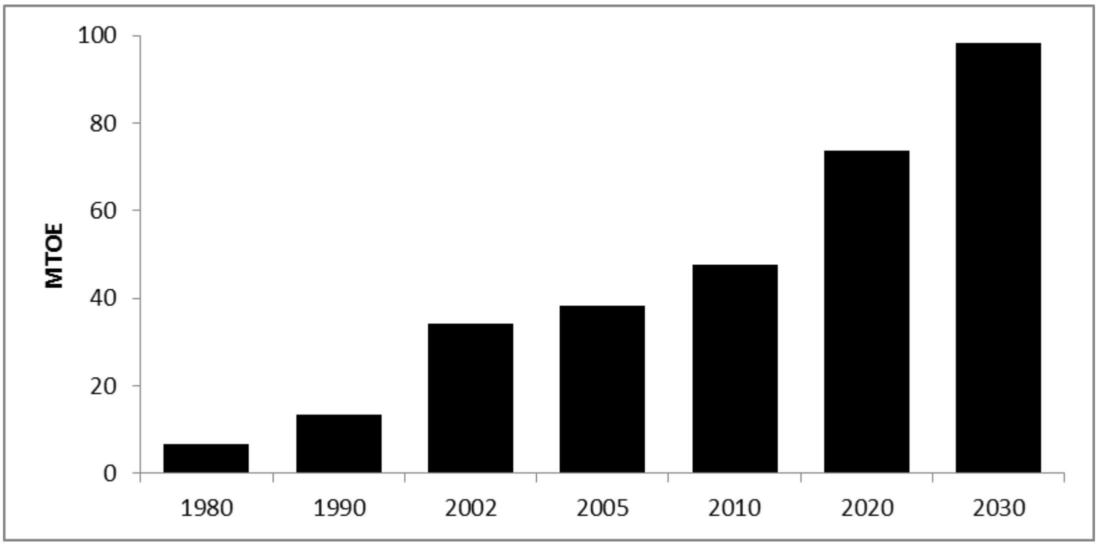

Figure 1.1: Trend of energy demand in Malaysia from 1980-2030 (Shuit *et al*. 2009).

There are several types of renewable energy such as solar, wind, ocean and thermal technology. Apart from that, biomass is identified as one of most promising resource of renewable energy. There are several advantages of producing bioenergy from crops origins in terms of economy, environment and energy security (Balat, 2011). Economically, renewable energy will contribute to the sustainability, fuel diversity and reducing the dependency on imported petroleum. The application of bioenergy would bring less harm to the environment, where the emission greenhouse gases can be reduced, while contributing to higher combustion efficiency with the reduction of air pollution. Bioenergy will benefit the energy security of a nation, as it would reduce the dependency of fossil fuel, in term of renewability and availability.

1.2 Bioethanol as Renewable Energy

Bioethanol, (C_2H_5OH), an example of renewable energy can be derived from several feedstocks. Bioethanol is mainly used for transportation. Apart of that, bioethanol can be used to substitute lead as an oxygenating additive, hydrogen carrier for fuel cells and also to generate electricity. Besides that, bioethanol can be employed in other industries such as pharmaceutical, cosmetics, beverages as well as for industrial uses.

According to Balat, (2011), bioethanol feedstock can be divided into three categories: sucrose-rich crops, starch rich corps and lignocellulosic materials. The first two categories were known as the first generation of bioethanol which employed the usage of food resources such as corn and sugarcane as raw material. The first bioethanol generation were seen to show promising replacement to gasoline production which is used mainly in the transportation sector. Countries such as United States and Brazil, bioethanol were commonly used as fuel (RFA, 2014). Despite that, there is a rising concern over the fact that bioethanol production from the first generation would affect the food supply. In addition, the production of bioethanol is not sufficient as an alternative to supplement the enormous amount of fossil fuel consumed worldwide each year. Hence, extensive research with the aim of finding promising, inedible feedstock for an alternative is carried out in decades. In this regards, lignocellulosic-based biofuel which was also classified as the second generation has shown great potential to be exploited as the non-edible raw material for bioethanol production.

Based on statistics reported by Litch (2014), the total production of ethanol fuel for 2014 was 23.4 billion gallons with the United States as the main producer (13.3 billion gallons). Figure 1.2 shows the world ethanol fuel production from 2009 until 2014. There is a slight increment of total ethanol production from 2012-2014 for United States.

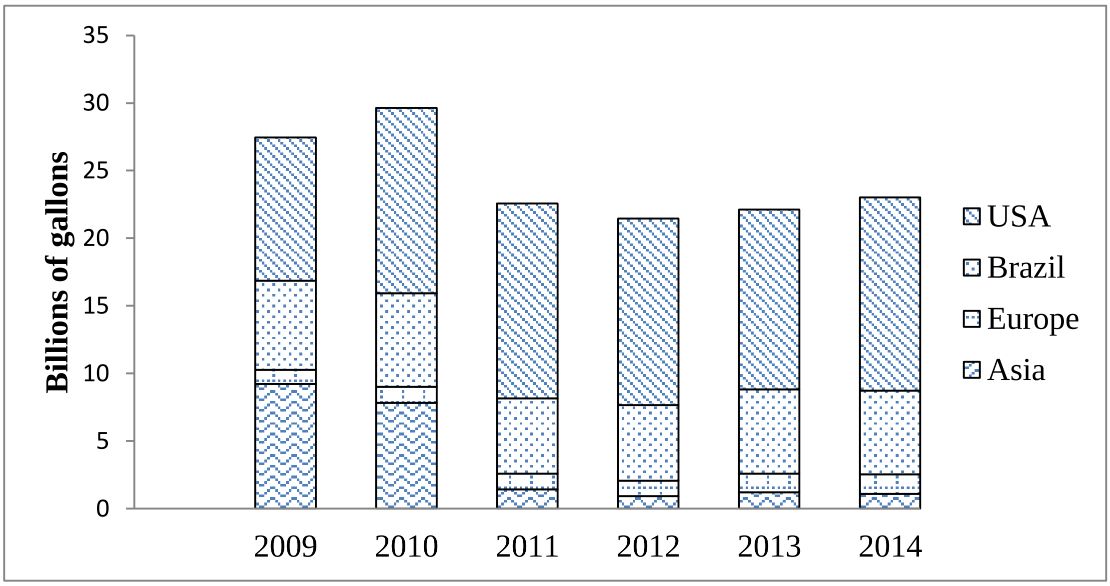

Figure 1.2: World ethanol fuel production (Adapted from Litch, 2014)

1.3 Empty Fruit Bunch (EFB) as Biomass Feedstock for Bioethanol Production

Agriculture waste is one of the main components of the second generation bioethanol. Malaysia, as the world's second largest producer as well as the world's largest exporter of palm oil, produced tremendous amount of oil palm biomass each year. According to statistic data obtained from MPOB (2014), the area covered for oil palm planted area by December 2013 throughout Malaysia was recorded as 5.3 million hectare. It is also reported that amount of crude palm oil produced for the second quarter of 2014 was 6.9 tonnes (MPOB, 2014). In correlation with these two statistical values, an enormous amount of waste was generated. EFB (wastes generated during the production of palm oil production) comprised of lignocellulosic materials. Due to their lignocellulosic structure, EFB has been identified as a potential resource for biofuel production, hence providing an alternative to cope with the increasing energy demand.

1.4 Problems Statement

Even though an enormous amount of EFB was generated from the palm oil production, the usage of its residue is not being fully exploited. The residues is usually burnt or used as feedstock for boilers to generate energy that could lead to air pollution problems. Hence, converting this biomass into renewable energy would contribute to the field of waste minimization and utilization. Furthermore, the development of alternative energy nowadays is towards sustainable energy that viable economically and environmentally. There is much attention to use a non-food source for renewable energy which is known as a second generation biofuel (Gnansounou and Dauriat, 2010).

There is much research has been done on converting palm biomass to bioethanol. However, the commercialization of the process is still discouraging as there is limited or known proven industrial scale technology developed from laboratory scale. Due to their recalcitrant structures, the process involved multiple stages until the pure ethanol is produced. This has become a challenge of bioethanol production from biomass.

To convert cellulosic materials to bioethanol, pretreatment is the most important process which would influence the overall production of bioethanol. Pretreatment of lignocellulosic material was required to break the lignin-hemicellulose-pectin complex and disturbed the crystalline structure of cellulose. Ideally, pretreatment process should have the following criteria: (i) maximum fermentable carbohydrate (ii) maximum valuable by-product but minimum inhibitory product, (iii) low environmental effect, (iv) required minimum downstream processing, (v) low energy requirement (Zheng *et al*., 2009). These criteria will be the basis of pretreatment selection of this work with the intention to make the process is more attractive to the industry.

There is several pretreatments process available such as physical, chemical and biological. This pretreatments process is often referred as a single pretreatment applied onto biomass. Each pretreatment has its own advantages and disadvantages. Physical treatment such as grinding, milling, chipping and shredding usually increased the positivity of the surface area or reduced the degree of polymerization and cellulose crystallinity. However the efficiency of the process was not often satisfactory together with physical treatment alone. On the other hand, chemical treatment, acid and alkaline treatments are usually employed. Acid pretreatment has a solubilizing effect onto hemicellulose while alkaline pre-treatment was known to be effective in the delignification process (Marcotullio *et al.*, 2011). The main drawback of the chemical pretreatment is that the toxicity of the chemical used would sometimes cause an inhibitory factor in subsequent process (hydrolysis and fermentation). The use of high acidity or basic chemical would lead to multiple steps of neutralization. Thus, it is required to find the most suitable method that could overcome or reduce the severity of weaknesses for selected pretreatment. The novelty of this work is developing the sequence process of combining two different pretreatment using cheap and available tool such as commercial pressure cooker and dilute acid pretreatment (DAP) which provide low cost and effective pre-treatment to enhance fermentable sugar yields for bioethanol production.

1.5 Objective for the Study

In general, this work is aimed to improve the performance of pretreatment process of converting palm biomass into fermentable sugars for bioethanol production by introducing the sequence of incorporated technique: low pressure steam heating (LPSH) and dilute acid pretreatment (DAP). With this aim, the objectives of this study are divided as follows:

i. To carry out the preliminary study of different pretreatment method as a basis of selection.

ii. To investigate the performance of LPSH and the combination with DAP.
iii. To study the surface morphology of the selected pretreatment in order to observed ruptured structure and crystallinity using Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD).
iv. To verify the fermentability of the sugars obtained from the combined pretreatment.

1.6 Scope of Study

To achieve the stated objectives of this study, the scope of experiment was limited as follows:

i. Compositional analysis of (EFB) by analyzing its chemical properties and elemental composition.
ii. Comparison of the effect of three common chemical pretreatment methods (dilute acid, lime and ammonia) of EFB on sugar and bioethanol production in the preliminary study.
iii. Investigate the effect of pressurized vessel on efficiency pre-treatment
iv. Investigate the effect of several parameters such as residence time, acid concentration, and pressure in the combination method.
v. Morphological and crystallinity changes determined by Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD) analysis.
vi. Verification of ethanol content using simple fermentation process through High Performance Liquid Chromatography (HPLC).

The characterization of chemical properties of empty fruit bunch was carried out by adapting the procedures from the laboratory analytical procedures (LAPs), National Renewable Energy Laboratory (NREL) (1998). The analysis consisted of total lignin content, extractive content, glucose content using Bioehringer

Mannheim/R Biopharm and xylose content using Megazyme xylose kit (Asli *et al*., 2012).

Preliminary study was conducted to compare the best pre-treatment technique between three pretreatment comprised of lime, dilute acid and ammonia in order to determine the best methods to produce fermentable sugars. The best pretreatment techniques during preliminary steps were incorporated with low pressure steam heating (LPSH) as a combination treatment onto the EFB sample. Further studies were done for combined pretreatment to investigate the effect of residence time (5-45 minutes), different concentration of dilute acid (1-4%) and low pressure (5-10 psi) regarding the saccharification yields.

Morphological study was carried out to observe any changes into surface of EFB samples before and after pretreatment. This study was carried out using SEM while changes in crystallinity was determined using XRD. Verification of fermentable sugars obtained from the hydrolysis stages was done by simple fermentation process, using instant Baker's yeast as the ferment agent. 10% of the instant baker's yeast (*Saccharomyces cerevisiae* strain) and several drops of oil were added into a conical flask. The fermentation process was carried out in incubator at 45°C for 72 hours. However, this is not the main scope of this study. The fermentation process was done in order to verify the success of pretreatment in order to produce fermentable sugar to obtain ethanol.

Ethanol determination was carried out using High Performance Liquid Chromatography (HPLC). The result obtained was qualified and quantified the amount of ethanol in the sample.

1.7 Significant of Study

In order to make the process attractive and adapted onto larger scale, it must be of economic value consisting of low production cost but able to produce higher yield. However the main challenge for this route was the complex structure of lignocelluloses in palm biomass itself. In order to separate the cellulose from other components, pre-treatment of oil palm biomass was required to achieve significant level of fermentable sugars before fermentation process. Effective pretreatment was necessary in order to break the natural structure of lignocellulosic prior to the conversion of cellulose to glucose. Hence the motivation of this study was to provide a low cost and effective pre-treatment to enhance fermentable sugar yields for bioethanol production. With this regards, the employment of pressure cooker as an existing equipment which is simple and effective with combination of dilute acid-pre-treatment was evaluated. The utilization of pressure cooker was an economically feasible as it is available tool in oil palm mill. In addition, the process sequence developed from this study can be potentially adapted into larger scale like pilot scale for further study. The synergy between combined pretreatment was expected to increase the enhancement of sugar produced.

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