# Fermentable Sugars from Palm Empty Fruit Bunch Biomass for Bioethanol Production

U. A. Asli, H. Hamid, Z.A. Zakaria, A. N. Sadikin, R. Rasit

*Abstract*—This study investigated the effect of a dilute acid, lime and ammonia aqueous pretreatment on the fermentable sugars conversion from empty fruit bunch (EFB) biomass. The dilute acid treatment was carried out in an autoclave, at 121ºC with 4% of sulfuric acid. In the lime pretreatment, 3 wt % of calcium hydroxide was used, whereas the third method was done by soaking EFB with 28% ammonia solution. The EFB biomass was then subjected to a two-stage-acid hydrolysis process. Subsequently, the hydrolysate was fermented by using instant baker's yeast to produce bioethanol. The highest glucose yield was 890 mg/g of biomass, obtained from the sample which underwent lime pretreatment. The highest bioethanol yield of 6.1mg/g of glucose was achieved from acid pretreatment. This showed that the acid pretreatment gave the most fermentable sugars compared to the other two pretreatments.

*Keywords*—Bioethanol, biomass, empty fruit bunch (EFB), fermentable sugars.

## I. INTRODUCTION

BIOETHANOL from palm oil biomass is a promising alternative biofuel as it is derived from plentiful and renewable non-food. In Malaysia, at least 60 million tons of palm oil residue is generated every year due to large-scale plantation and palm oil processing. However, the commercialization of this lignocellulosic bioethanol is still not optimum. The operating cost is considered too high, which has hindered the industry's players to produce bioethanol commercially.

Theoretically, lignocellulosic biomass is mainly built up from a complex matrix with the constituent of three polymers which are cellulose, hemicelluloses and lignin. These structures are considered "stubborn" to degrade into valuable matters. Typically, early treatment is needed to reduce the recalcitrant structure and disrupt the lignin from the cellulose matrix. This allows the locked sugar between the highly crystalline polymers to be obtained by chemical or biological conversion, either by acid or enzymatic hydrolysis.

A physical treatment, such as grinding, milling, chipping and shredding has usually positively increased surface area or reduced a degree of polymerization and cellulose crystallinity. However, the process efficiency is not very often satisfactory with the physical action alone. A combination with chemical pretreatment could increase the accessible area or digestibility of biomass prior to the hydrolysis process.

Numerous acids, including sulfuric acid, nitric acid, hydrochloric acid, phosphoric acid and acetic acid have been proven effective for cellulosic conversion [1]-[6]. The acid condition is either in concentrated solution (10 to 30%) or diluted form (2 to 5%) [7]. Acid pretreatment has a solubilizing effect on the hemicellulose, but lignin and cellulose remain intact. Compared with dilute acid pretreatment, concentrated acid is powerful and effective, but is lacking in other aspects in terms of toxicity, corrosive and hazardous, and requiring costly reactors that are resistant to corrosion [8]. Dilute acids have received more interest due to their moderate process condition requirement, and mostly applied for the subsequent process, either a two-stage acid hydrolysis or enzymatic hydrolysis.

Besides acid, alkaline solution is also commonly employed in the biomass fractionation process such as sodium hydroxide, calcium hydroxide, potassium hydroxide and aqueous ammonia [9]-[11]. This alkaline pretreatment is known to be effective in delignification by the action of lignin structure disruption, including a large amount of hemicellulose. The result is a bond between lignin and other carbohydrate parts that can be broken down into hetero-matrix simpler carbohydrates, increasing the reactivity parts of polysaccharides [8].

The selection of pretreatment is crucial as it is the most costly process in cellulosic bioethanol production. An ideal pretreatment process must have the following criteria: (i) maximum fermentable carbohydrate (ii)) maximum valuable by-product but minimum inhibitory product, (ii) low environmental effect, (iii) required minimum downstream processing, (iv) low energy requirement [8]. Above all, an economical scheme is more preferable for the process to be commercialized. Thus, this present work has chosen three simple methods, which are dilute acid, lime and ammonia aqueous for the EFB biomass pretreatment. This work studied the relationship of pretreatment processes with their fermentable sugars production and bioethanol production.

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## II. EXPERIMENTAL PROCEDURE

### A. EFB Biomass Sample Preparation

The EFB sample was taken from Felda Simpang Waha Oil Palm Factory located at Kota Tinggi, Johor. The sample was dried using convection oven at the temperature of 105°C for one week. The sample was cut into between 0.1 to 0.5mm size pieces.

World Academy of Science, Engineering and Technology
International Journal of Chemical and Molecular Engineering
Vol:7, No:12, 2013

### B. Dilute Acid Pre-Treatment

An amount of 15g dried EFB is mixed with 300ml of 4% sulfuric acid ($H_2SO_4$) and placed in autoclave for 1 hour at a temperature of 121 +/- 3°C. After that, the mixture was allowed to cool at room temperature before being filtered 4 to 5 times until the filtrate became clear. The filtrate was then dried at 45°C for 48 hours.

### C. Lime Pre-treatment

In this method, an amount of 15g of dried EFB was mixed with 5g of Calcium Hydroxide in the 150ml of distilled water. The mixture was mixed thoroughly with a glass rod to ensure an even distribution of lime and water. The slurry was heated to boil for 2 hours with occasional stirring. After that, the mixture was allowed to cool at room temperature. The pH of the mixture was adjusted within a range of 5.5 to 6 by adding dilute glacial acetic acid. The volume ratio of glacial acetic acid to distilled water used is 1:2. In addition of 300ml of distilled water was added into the slurry while stirring for 15 minutes. The slurry was then filtered 4 to 5 times using a filter funnel until the filtrate become clear. After being completely washed, all the washed filtrate was dried at 45°C for 48 hours.

### D. Ammonia Aqueous Pretreatment

First, a solution of 21% of ammonia aqueous was prepared. An amount of 20g empty fruit bunch was mixed with 120ml aqueous ammonia by stirring with a glass rod. Then, the mixture was left in a water bath at a temperature of 60°C for 6 hours. Afterwards, the pretreated EFB sample was filtered using the filter funnel. The whole process was repeated until the filtrate became clear. After the sample was completely washed, all the biomass was transferred and dried at 45°C for 48 hours.

### E. Analysis of Extractive Content

The content of total lignin and recovered sugars (in a form of glucan and xylan) of the prepared biomass samples was determined using a two-stage acid hydrolysis procedure following the NREL standard procedure "Determination of Extractives in Biomass Laboratory Analytical Procedure (LAP)" [12]. At first, an amount of 1 gram of the sample was added to 15mL of 72% (w/w) $H_2SO_4$, and stirred with a glass rod for 1 minute until the sample was thoroughly wetted. The mixture was hydrolyzed for 2 hours at room temperature. The hydrolyzate was then diluted to 3% by adding distilled water. Subsequently, the hydrolysate was heated to gently boil and reflux for 4 hours, and finally cooled down at room temperature for analysis.

The total lignin content is measured from a summation of insoluble and acid-soluble lignin content. The determination of insoluble lignin was done following the Klason Method. The insoluble lignin was determined from the average percentage of extractives in the sample on a dry weight basis. The acid soluble lignin content was determined in a hydrolysis solution by using a spectroscopy at a wavelength of 205nm.

To determine the glucose and xylose contents, a Glucose Bioehringer Mannheim/R Biopharm test kit (Germany), and a D-xylose assay kit (Megazyme International Ireland) were used, respectively. The cellulose content was calculated by subtracting between the glucose contents of saccharides and the one from the total glucose content from the hydrolysis method. The xylan content was calculated from the amount of xylose, under the assumption that xylan is a linear polymer of b-1,4-linked xylose residues.

Morphological and structural analysis of all samples was obtained from the Scanning Electron Microscopic imaging technique.

### F. Fermentation

First, the pH condition of a 50ml of hydrolysate from the pretreatment procedure was adjusted to pH 5. Afterwards, an amount of 5 gram instant baker's yeast (*Saccharomyces cerevisiae* strain) was added into a conical flask. Two to three drops of oil were added into the mixture to minimize the exposure to air. The mixture was incubated for 72 hours in the incubator at a temperature of 40°C. The mixture of hydrolysate was filtered after 3 days and the filtrate was analyzed by Gas Chromatography to determine ethanol content. The yield of ethanol obtained was calculated by the ratio of ethanol determined in solution to the glucose used in the solution.

## III. RESULTS AND DISCUSSION

Fig. 1 shows the effect of different pretreatment methods on sugar yield after the acid hydrolysis. EFB biomass sample without pretreatment was also examined as a basis of comparison. The result shows that the highest glucose recovery of 890 mg/g of biomass was achieved from the lime pretreatment method. This result is comparable with a similar study [13]. It can also be seen that both aqueous ammonia and the dilute acid pretreatment method had also increased the glucose conversion. The glucose yield obtained was 44 and 51% higher using ammonia and acid, accordingly, relative to the untreated sample. Surprisingly, the untreated sample had been hydrolyzed to xylose, with 695mg production per g of EFB biomass, showing that the acid hydrolysis condition used was efficient enough to convert the untreated EFB fibers into xylose.

In terms of lignin degradation, it could be seen from Fig. 2, all the pretreatment did not influence the lignin removal from EFB fibers. As expected, under mild conditions, it is difficult to reduce lignin from the fiber as lignin is the most stable component in lignocellulosic structure.

World Academy of Science, Engineering and Technology
International Journal of Chemical and Molecular Engineering
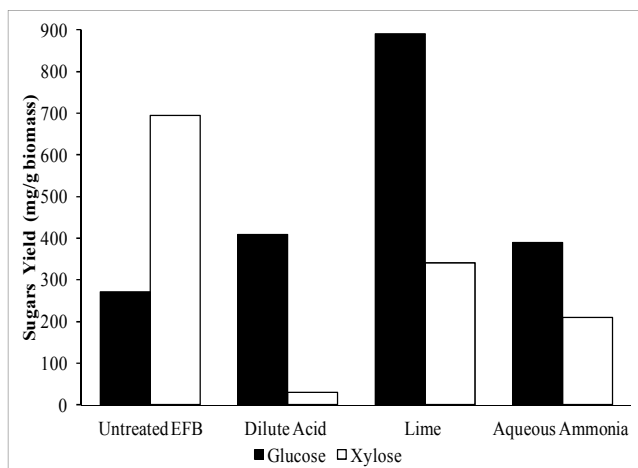Vol:7, No:12, 2013

Fig. 1 Effect of different pretreatments and to the hydrolysis yield
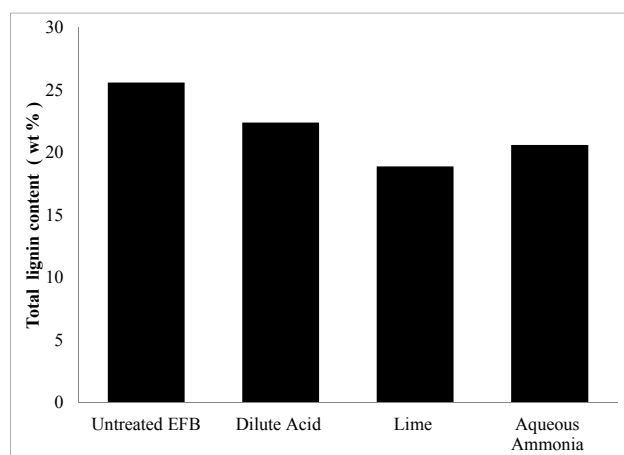(glucose and xylose) compared with untreated EFB sample



Fig. 2 Amount of total lignin degraded from EFB biomass with
different pre-treatment and without pretreatment

To observe the structure changes, the morphology of the
EFB sample from all different pretreatments was compared
with the original sample (without pretreatment) using the SEM
technique. All the SEM images are shown in Fig. 3. In general,
it could been observed that the morphology of EFB sample
treated with all pretreatments (Figs. 3 (b) to (d)) was disrupted
and shattered in comparison with the original structure (Fig. 3
(a)). The most severe structure disruption is observed on the
SEM images of the lime pretreated sample (Fig. 3 (c)). This is
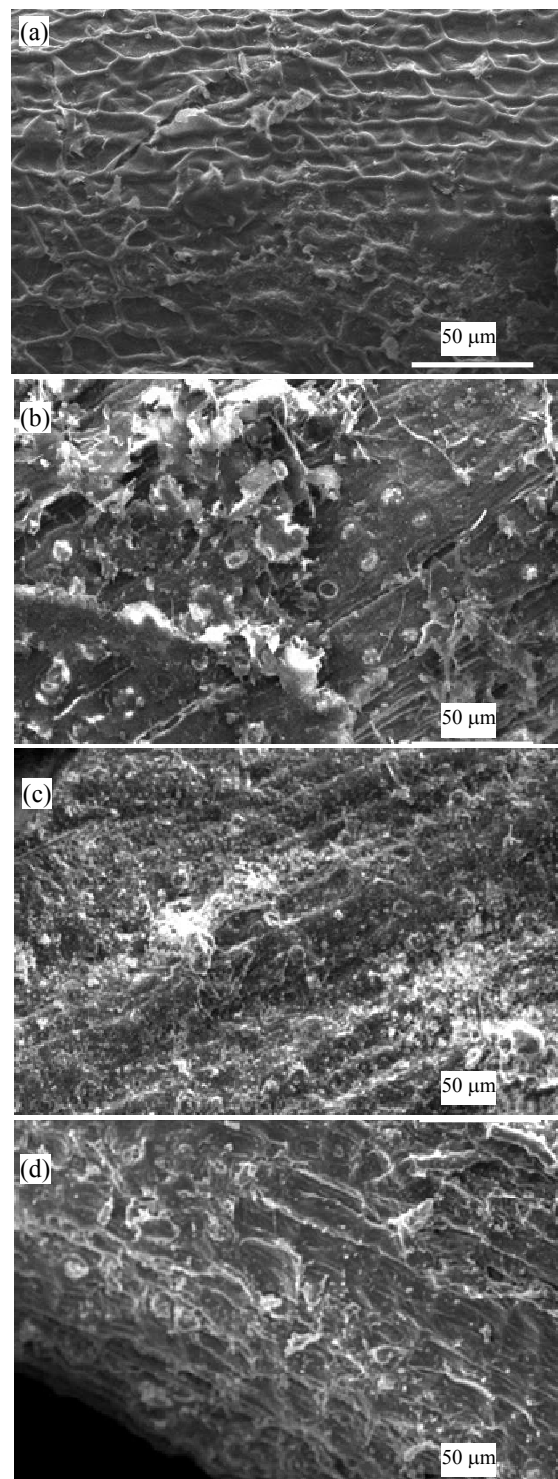actually consistent with physical changes observed after the
pretreatment.



Fig. 3 SEM images of EFB biomass surface from : (a) untreated
sample; (b) dilute acid; (c) lime; (d) ammonia aqueous pretreatment

Although the lime pretreatment gave the highest glucose
recovery, the lowest ethanol yield (2.2mg/ g glucose) was
obtained. The hydrolysate from dilute acid treatment achieved
the highest ethanol yield of 6.1mg/g glucose. There was no
ethanol produced from the untreated biomass sample. This
could be due to the fact that the yeast strain used
(*Saccharomyces cerevisiae*) is xylose non-metabolize [14].

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The xylose content might inhibit the fermentation because the xylose can be converted into furfural:- the common inhibitor is glucose fermentation. Since the acid treatment produced the least xylose content, it reduced inhibitory effect on the fermentation process to ethanol.
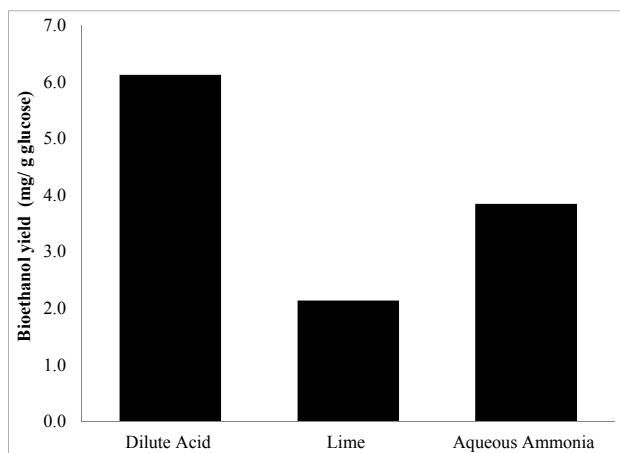


Fig. 4 Ethanol yield from sugars recovered via different pretreatment

## IV. CONCLUSIONS

The motivation behind this work is to select an efficient and cost effective method for bioethanol production so that it is more reasonable to be commercialized.

Results show that among the three methods used, the lime method gave the highest sugars recovery, relatively three times higher than the recovery from the untreated sample. Also, from the SEM images, it is understood that the structure of EFB biomass is the most disrupted after it underwent the lime pretreatment. In terms of fermentability of the recovered sugars, it is found that the highest bioethanol yield was obtained from the biomass sample treated with dilute acid. This shows that dilute acid treatment is the most suitable for a subsequent fermentation process after acid hydrolysis. Although the ethanol yield is low, this is an interesting process since this method is simple, requiring only moderate conditions and yet sufficient to degrade EFB biomass into fermentable sugars.

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