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Pretreatment of Oil Palm Empty Fruit Fiber (OPEFB) with Aquaeous Ammonia for High Production of Sugar

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Abstract

Corncob Oil Palm Empty Fruit Bunch (OPEFB) is an agricultural residue that has the potential to become a good source for renewable feedstock for production of sugar. This work evaluated the effectiveness of aqueous ammonia as pretreatment at low (soaking, SAA) and elevated temperature (Pressurized Chamber) to deconstruct the lignocellulosic feedstock, prior to enzymatic hydrolysis. The ammonia pretreatments were compared against the standard NaOH method. The best tested Pressurized Chamber method conditions were at 100ºC with 3 hour retention time, 12.5% Ammonium hydroxide and 1:30 solid loading. The digestibility of the feedstock is determined with enzymatic hydrolysis using Cellic Ctech2 and Cellic Htech2. The sugars produced by Pressurized Chamber method within 24 hour of enzyme hydrolysis are similar to that produced by NaOH method which is 439.90 mg/ml and 351.61mg/ml, respectively. Compared with optimum SAA method (24 hour, 6.25% of ammonium hydroxide at room temperature), Pressurized Chamber method was capable of producing enhanced delignification and higher production of sugar upon hydrolysis. These findings were supported by the disappearance peak at 1732, 1512 and 1243 on Fourier Transform Infrared (FTIR spectrum) of treated OPEFB by Pressurized Chamber method. XRD determination showed reduced crystallinity of OPEFB (37.23%) after treatment by Pressurized Chamber, suggesting higher accessibility toward enzyme hydrolysis. The data obtained suggest that the Pressurized Chamber pre-treatment method are suitable for OPEFB deconstruction to produce high yield of sugar.

Peer-review under responsibility of the organizing committee of the Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 (MCLS 2015)

Keywords: Aqueous Ammonia pretreatment; Empty Fruit Bunch (EFB); Pressurized chamber; Bio-mass conversion

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Nomenclature

OPEFB	Oil Palm Empty Fruit Bunch
HPLC	High Performance Liquid Chromatography
SAA	Soaking in Aqueous Ammonia
XRD	X-Ray Diffraction Method
FT-IR	Fourier Transform Infra-red Sprectroscopy
SEM	Scanning Electron Microscope

1. Introduction

Complexity of lignocellulosic biomass is due to abundant of lignin, hemicelluloses and high degree of crystallinity of cellulose. Both cellulose and hemicelluloses are polymer of sugar thus it is suitable and potential source of fermentable sugars into products, such as bio-ethanol. Due to the complex structure, pretreatment are necessary to alter the structure of cellulosic biomass to be more accessible for enzyme hydrolysis. Ideal pretreatment goals are removal of lignin, reduce crystallinity of cellulose, modification of lignin structure and increase the surface area and porosity of lignocellulosic material. Thus enhance production of sugar by enzymatic hydrolysis[1,2].

Alkaline pretreatment have several advantages, especially in its capability for efficient for lignin solubilization. Alkaline pretreatment also known to cause swelling of the structure of fiber treated thus enlarge the internal surface area of cellulose and decrease the polymerization and degree of crystallinity of cellulose[2]. Studies shows alkaline pretreatment remove acetyl and various uronic acid in hemicelluloses that allow more accessibility of enzyme to the hemicelluloses and cellulose surface[3].

Therefore the aim of this study is to assess the effect of different ammonia-based alkaline pretreatment methods prior to enzymatic hydrolysis for the production of sugar.

2. Methods

2.1 Sample Collection and Preparation

Oil Palm Empty Fruit Bunch (OPEFB) was collected at Seri Ulu Langat Palm Oil Mills Sdn.Bhd. Selangor, Malaysia. The fiber was washed and dried until the moisture content reached 10-11%. The dried OPEFB was cut to 4-5 cm for efficient pre-treatment size. The fiber is stored at 4ºC for next usage[4].

2.*2 Chemicals & Materials*

Chemicals used for pretreatment are ammonia solution 25% (Merck), Sodium Hydroxide pellets (NaOH) purchased from Systerm Sdn Bhd,Selangor. Enzyme used are Cellic Ctech2 and Cellic Htect2 from Novozymes A/S Denmark High Performance Liquid Chromatography (HPLC) grade solvent for sugar analysis were acetonitrile HPLC Grade (Merck) and ultrapure water. Glucose, xylose and cellobiose (Sigma Aldrich, USA) were used as standards.

2.3.Pretreatment

2.3.1 Soaking in Aqueous Ammonia (SAA)

OPEFB was treated with ammonium hydroxide at room temperature with different concentration of ammonium hydroxide and soaking time. The experiment was designed by using Response Surface Methodology. The parameter have been chosen for analysis are S4 (6.25% NH_4OH, 24 Hour), S6 (13.13% NH_4OH, 14 Hour), S8 (20% NH_4OH, 24 Hour). The compositional analysis of samples was determine by refering to NREL method[5,6,7].

2.3.2 Pressurize chamber

The OPEFB was treated in closed pressurized chamber system. The temperature was set at 70ºC and 110ºC with solid loading 1:15 and 1:30 at 1 hour and 3 hour retention time.The samples were soaked and heated in closed system. The pressure was monitored and after the experiment was complete, the pressure valve was gradually open to relieved the pressure.The best pretreatment was at 110ºC at 1 hour and 3 hour retention time with 1:30 solid loading was proceed to the next stage analysis. The treated OPEFB was left overnight under fume hood to evaporate the residue of ammonia. The compositional analysis of samples was determine by refering to NREL method[5,6,7]

2.3.3 Sodium Hydroxide (NaOH)

NaOH pretreatment was conducted at 10% solid loading. The OPEFB was soaked in NaOH at 120ºC for 2 hour. The treated fiber then was washed with distilled water and left under fumehood for air dried. The dry treated OPEFB is kept sealed at 4ºC to ensure the moisture content not changed. The compositional analysis of samples was determine by refering to NREL method[5,6,7]

2.4. Enzymatic Hydrolysis

Enzymatic hydrolysis of treated OPEFB was conducted in citrate buffer medium at pH 4.8, 50±1ºC at 150 rpm agitation in incubatory shaker. The enzyme used was mixture of Cellic Ctech2 and Cellic Htech2 at ratio 1:1. Hydrolysis of enzyme was conducted for 24 hours. The production of monomeric sugar glucose and xylose was determine by HPLC, using sugar analysis column Purospher STAR NH_2 (Merck ,Germany) with evaporative light scattering detector (ELSD), PL-ELS 1000E. Mobile phase used was acetonitrile (HPLC Grade) : deionised water , 80:20 per volume. Flow rate was set at 1ml/min. Injection volume was set at 20 µl with 35 minute analysis duration time. Chromatogram was reported and analysed using Breeze Software (Waters,Milford,USA).

3. Results and discussion

3.1 Sugar Analysis

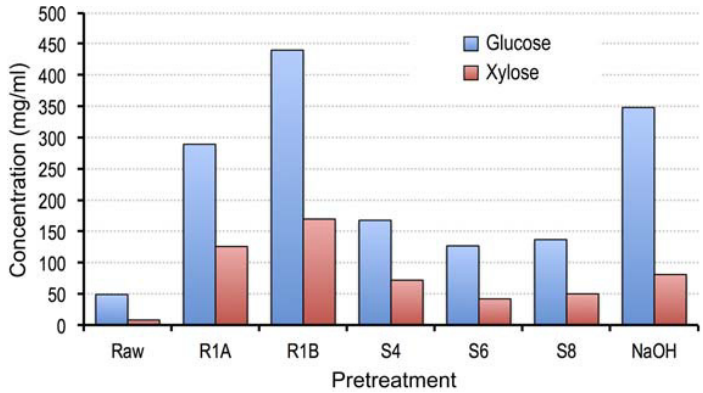

Fig. 1. Bar chart shows production of Glucose and Xylose for each pretreatment after enzymatic hydrolysis. S4 (6.25% NH_4OH, 24 Hour), S6 (13.13% NH_4OH, 14 Hour), S8 (20% NH_4OH, 24 Hour), R1a (100ºC, 1 Hour) amd R1b (100ºC ,3 Hour)

Fig. 1 shows the production of sugar (glucose and xylose) after enzyme hydrolysis of raw and treated OPEFB for 24 hours. Treated OPEFB show increase in production of glucose and xylose compared to raw OPEFB. The lower yields of sugar from raw OPEFB was due to high lignin, high hemicellulose and high crystallinity of cellulose that cause less production of glucose and xylose. OPEFB treated with NaOH act as bench mark as capability of NaOH as pretreatment in delignification and production of high sugar yield.

SAA pretreatment are mild pretreatment at room temperature. Treatments S4, S6 and S8 produce 168.58 mg/ml, 125.32 mg/ml & 134.89 mg/ml of glucose after 24 hours of enzymatic hydrolysis. Increase in time of pretreatment from 14 hours (S6) to 24 hours (S4&S8) does not have much significant increase in glucose and xylose production. Similarly SAA pretreatment with higher concentration of 20% ammonium hydroxide (S8) does not produce as high glucose and xylose as compared to SAA pretreatment with 6.25% ammonium hydroxide (S4). Both pretreatments took place for 24 hours.

In pressurize chamber pretreatment, increase in retention time from 1 hour to 3 hour shows significant increase in production of glucose from 290.28 mg/ml to 439.90 mg/ml. It was the same with production of xylose from pressurize chamber pretreatment, where the increase in retention time produce 126.57 mg/ml to 171.59 mg/ml. Pressurize chamber method produce the highest glucose and xylose production compared to SAA as may be due to presence of high pressure apply during the pretreatment cause the distruption of lignin matrix and reduce crytallinity of cellulose[8]. Distruption of lignin matrix and decrease in crytallinity improve the hydrolysis rate.

3.2 X-Ray Diffraction

Degree of Crystalinity of treated OPEFB was determine by using X-Ray Diffraction Method (XRD). The OPEFB was dried in an oven at 50ºC for 3 hours before the analysis. The samples was analysed by using Diffractometer (Bruker D8 Advaced, Spain) at 2θ of 10ºC to 50ºC and Cellulose (SigmaCell Type 20, Sigma Aldrich,U.S) was used as standard. Fig. 2 shows diffractogram of OPEFB before and after pretreatment.

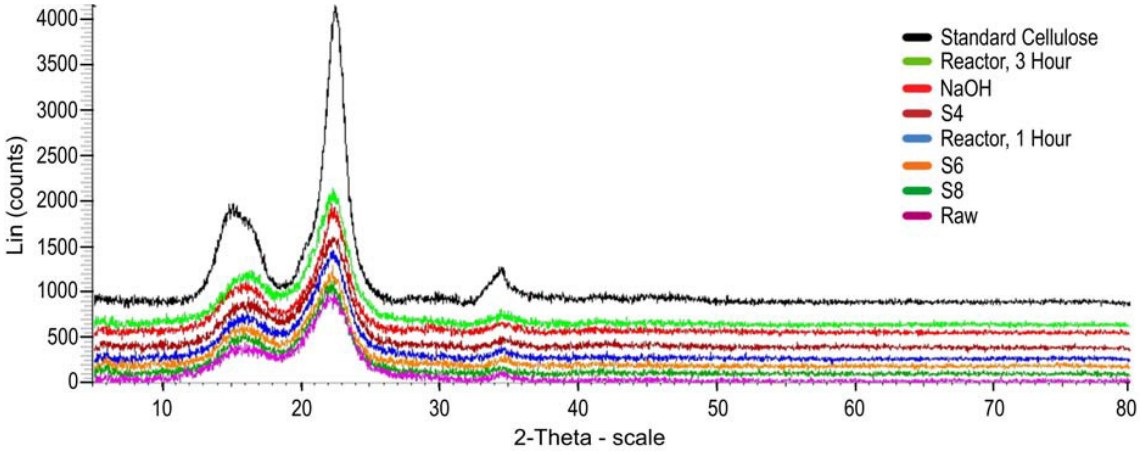

Fig. 2. X-Ray diffractogram of standard cellulose, pretretment of pressurized chamber 3 hour and 1 hour and pretreatment by soaking method

Table 1. Percentage of crystallinity of samples

Samples	Crystallinity, %
Raw	38.27
Sigmacell Cellulose (Standard)	66.42
NaOH	39.44
R1a (100ºC , 1 Hour)	42.91
R1b (100ºC , 3 Hour)	37.23
S4 (6.25% NH_4OH, 24 Hour)	38.69
S6 (13.13% NH_4OH, 14 Hour)	42.57
S8 (20% NH_4OH, 24 Hour)	39.23

Table 1 shows the % crystallinity of the OPEFB before and after the pretreatment. Cellulose from SigmaCell was used as standard. The diffractogram of treated and non-treated OPEFB shows similar trend with standard Cellulose (SigmaCell) shows presence of cellulose. OPEFB treated with Pressurize chamber for 3 hour (R1b) have the lowest degree of crystallinity 37.32%. This may be due to the breakdown of cellulosic hydrogen bond by the impact of

pressure during the pretreatment[8]. Low degree of crystallinity will increase the efficiency of enzyme hydrolysis[9]. This have been shown in high production of sugar by R1b (439.90 mg/ml). The slight increment in percentage of crystalinity is may be due to removal of amourphous hemicellulose and lignin thus expose the structure of the cellulose[10].

3.3 Fourier Transform Infra-red Sprectroscopy (FTIR)

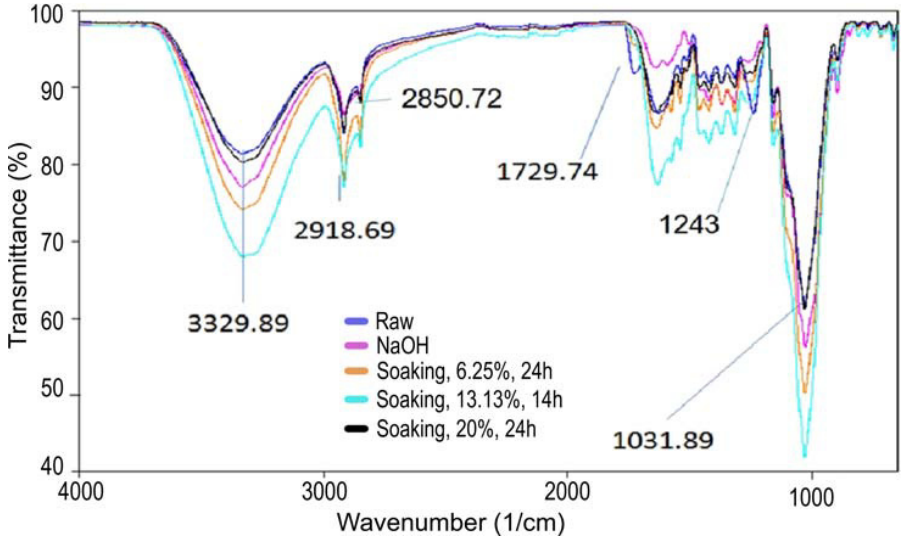

Fig. 3. FTIR spectrum of Raw, NaOH pretreatment and Soaking pretreatment

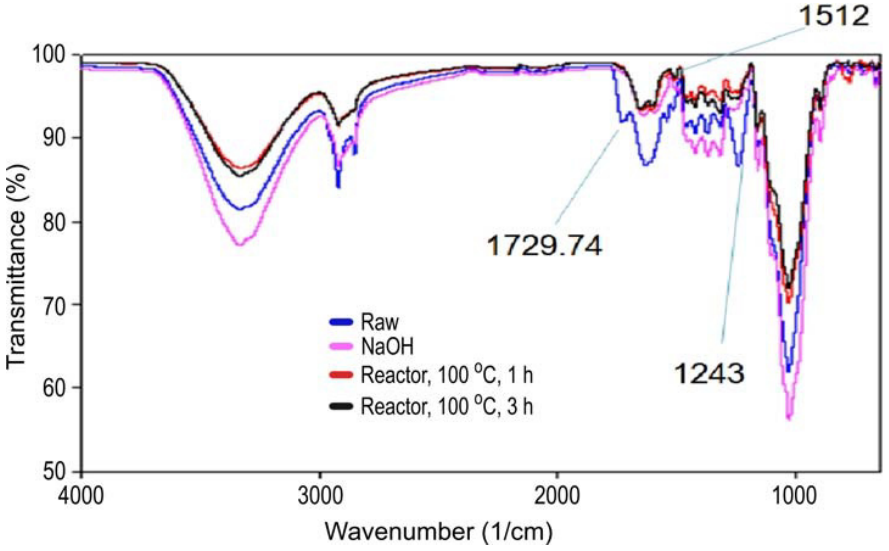

Fig. 4. FTIR spectrum of Raw, NaOH pretreatment and Pressurize pretreatment

The structural change of OPEFB was analyzed by FTIR spectra to detect loses of functional group of several compound. The results were shown in Fig. 3 and Fig. 4. Fig. 3 shows FTIR spectrum of Raw, NaOH pretreatment and Soaking pretreatment. Peak 3329.89 cm^{-1} represent hydrogen bonded (O-H) stretching absorption[11]. Stretching

at this particular region shows exposure of the cellulose structure. IR spectra at 1729.74 was assigned as C=O acetyl group of hemicelluloses ester or carbonyl ester of *p*-coumaric lignin[8,10]. In this peak probably exists linkage between hemicelluloses and lignin[11]. Fig. 4 shows treatment by soaking method (S4 and S6) shows slightly change in lignin and hemicelluloses content while soaking with 20% NH_4OH for 24 hours cause loss in functional group of lignin may be due to high concentration of NH_4OH. Pressurize chamber method R1a and R1b also shows similar trend loss in functional group at peak 1729.74 cm^{-1} similar with NaOH pretreatment. The loses of lignin functional group may be due to pressure apply during pretreatment for R1a, R1b and NaOH. Absorption at peak 1512 cm^{-1} represent aromatic stretching (C=C) from lignin[10,11]. Results show loss of aromatic functional group for NaOH pretreatment, R1a and R1b. No significance loss for Soaking pretreatment and Raw. Removal of C-O-C functional group of aryl-alkyl ether in lignin also was observed in NaOH pretreatment, R1a and R1b at intensity 1243 cm^{-1}. Removal of this 3 particular peak shows possible delignification for pressurize chamber, shows similar trend with NaOH pretreatment.

3.4 Scanning Electron Microscope (SEM).

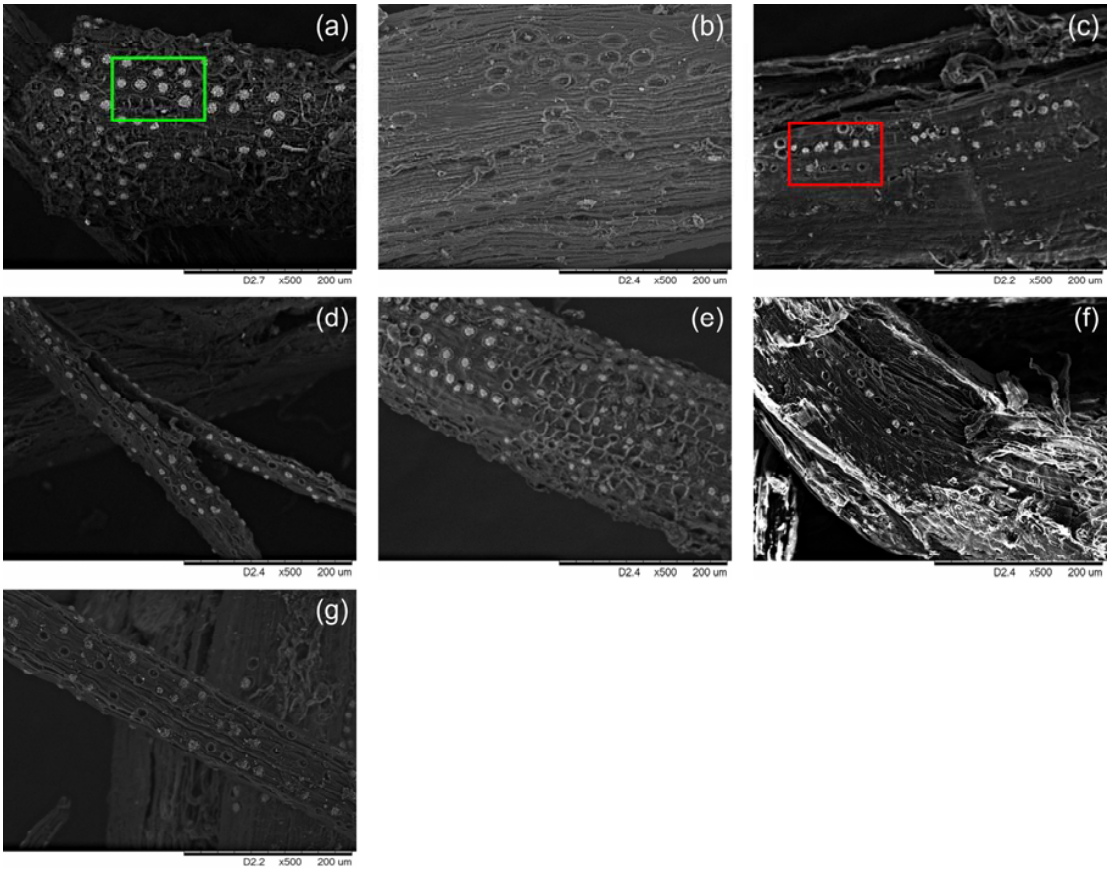

Fig. 5. SEM micrograph of (a) Raw OPEFB; (b) NaOH pretreatment; (c) S4 pretreatment; (d) S6 pretreatment; (e) S8 pretreatment; (f) R1a pretreatment; (g) R1b pretreatment. Examples of silica bodies and cavity spots are shown in green and red boxes, respectively.

The structural modification and physical changes in OPEFB were observed using SEM. Fig. 5 shows photomicrograph of Raw OPEFB and Treated OPEFB. Basically, the structures of OPEFB are smooth and consist of rigid and waxy surface. High degrees of crystallinity of the cellulose are contributed from the presence of silica body embedded on the surface of the plant. Pretreatment function to remove the presence of the silica body and expose the lignocellulosic structure to enhance and increase hydrolysis rate in production of sugar.

Fig. 5a shows raw OPEFB (untreated) consist of silica body and smooth surfaces. Most of the pretreatment involve help to remove silica and expose the cavities. Fig. 5b, NaOH treated OPEFB shows most loss of silica bodies and exposure of cavities. These have been proove by high sugar production and loss of lignin functional group from FTIR analysis. Presence of lignin will wrap the cellulose bundles and provide smooth area on the surface of the cellulose bundle[2]. Soaking pretreatment do not shows any significance different ($p>0.05$) in removing of silica bodies (Refer to Fig. 5c, 5d and 5e). Changes in the bundle structure can be seen in Fig. 5e because the usage of high concentration of Ammonium Hydroxide (20%). The most destructive structure can be seen at Fig. 5f and 5g as during pretreatment there are presences of pressure. In Fig. 5f and 5g also shows less presence of silica bodies and more cavities. This have been prove by loss of lignin functional group in FTIR analysis and high production of sugar.

4. Conclusion

SAA and Pressurize Chamber pretreatment were shown to be effective as pretreatment. SAA under optimal condition 6.25% NH4OH for 24 hours produce 168.58 mg/ml of glucose while optimal condition for Pressurize Chamber pretreatment 100ºC for 3 hours produce 439.90 mg/ml of glucose after enzyme hydrolysis. The glucose produce by R1b was slightly higher than glucose produce by NaOH pretreatment 351.51 mg/ml. XRD results shows R1b have low degree of crystallinity 37.23% allow higher production of sugar. Compared to SAA pressurized chamber method able to increase delignification as shown in FTIR spectra absence of functional group represent lignin at intensity 1729.74 cm^{-1}, 1512 cm^{-1}, and 1243 cm^{-1}.

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