

Factors Affecting Delignification of Oil Palm Empty Fruit Bunch by Microwave-assisted Dilute Acid/Alkali Pretreatment

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Microwave-assisted dilute acid/alkali pretreatment is an efficient and rapid method of removing lignin and hemicellulose, however, the optimized parameters for the maximum efficiency have to date not been presented in the literature. The purpose of this study was to determine those conditions by examining the effects of three factors: microwave power, temperature, and time on delignification in microwave-assisted dilute acid/alkali pretreatment. For the control condition of conventional pretreatment (CP), empty fruit bunches (EFBs) were soaked in 2.5 M NaOH for two hours in the autoclave. In the experimental condition, EFB were first soaked in dilute sulfuric acid with conventional autoclave heating, which removed 90% of their hemicellulose. The acid-treated EFBs were then soaked in 2.5 M NaOH solution and microwaved at different conditions: microwave power (700 - 1000 watts), time (60 - 90 min), and temperature (80 -110 °C). The amount of acid-insoluble lignin was determined by Klason method. Microwave-Alkali (Mw-A) pretreatment was modeled until it attained maximum delignification. More than twice the rate of delignification that is, 71.9% was attained with microwave-assisted alkali/acid pretreatment of 900 W microwave power at 110 °C for 80 min compared to 34.6% with conventional pretreatment.

Keywords: Microwave-alkali (Mw-A); Microwave-assisted dilute acid/alkali pretreatment; Oil palm biomass; Empty fruit bunches (EFB); Cellulose; Hemicellulose; Lignin

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INTRODUCTION

As the world's second largest producer of palm oil, Malaysia generates approximately 59 million tons/year of lignocellulosic biomass, comprised of empty fruit bunches (EFBs), oil palm trunks, oil palm fronds, and palm kernel shells (Chen *et al.* 2011). Lignocellulosic biomass is considered to be the world's most abundantly available biomass and it originates from plants as well as from industrial, agricultural, and forest residues. Pretreatment yields fermentable sugar from which industrially important compounds are produced. Different types of microorganisms like bacteria and fungi are used to degrade lignocellulosic biomass into glucose monomers (Kumar *et al.* 2008). Current studies show that lignocellulosic biomass, including oil palm, has the potential to produce industrially important compounds such as succinic acid (Akhtar *et al.* 2014).

Microwave irradiation can be used to accelerate the pre-treatment of this abundant resource if the substrate or solvent has the ability to absorb microwave/electromagnetic

energy and effectively convert it into heat energy. Molecules having a dipole moment try to align themselves with the oscillating electric field produced by microwave irradiation, leading to rotation. In solid and liquid phase, the rotation produces molecular vibrations that produce heat resulting in an increase in the sample temperature. When microwave irradiation is directed toward the substrate, some energy is absorbed, some energy is reflected while a part is transmitted through the surface. Microwave irradiation has two advantages. First, it has the potential to attain uniform heating despite the thickness of the target material and second, it can reduce reaction time from days and hours to minutes and seconds (Leonelli and Mason 2010).

Over the past decade, researchers have explored microwave-alkali (Mw-A) pre-treatment of lignocellulosic material as an alternative to conventional heating. Applications of this pre-treatment have been reported on rice straw (Zhu *et al.* 2005; Zhu *et al.* 2006; Ma *et al.* 2011), switchgrass (Hu *et al.* 2008), and EFB fiber (Hamzah *et al.* 2009). One of the several benefits of this new method as applied to EFB is the much higher removal of the silicon found in lignocellulosic fiber, as documented in a recent EFB morphology study (Hamzah *et al.* 2009). The presence of silica in the cell wall act as a barrier in the enzymatic digestibility and SSF system. Thus, its removal increased the enzyme digestibility of the EFB (Rezanka and Sigler 2008). Another benefit of sequential acid/alkali pretreatment is its greater efficiency in extracting cellulosic content. A more recent study (Kim *et al.* 2012) demonstrated that, after dilute sulfuric acid removed the maximum amount of hemicellulose and concentrated NaOH removed 90% of lignin, 10% (w/v) of EFB was hydrolyzed with an efficiency of 83.9%, resulting in 70.8 g/L glucose and 1.8 g/L xylose in the hydrolysate.

Dilute acid pretreatment with 1.51% concentration of H₂SO₄ was performed at 161.5 °C for 9.44 min, giving a glucose yield of 85.5%, higher than in any other commonly investigated feedstock. By contrast, dilute alkaline solution showed limited outcome, probably because of high lignin content (Chiesa and Gnansounou 2014).

Although all the pretreatment methods can disrupt the crystalline structure of lignocellulose, pretreatment selection is very much dependent on the type of lignocellulose and its structure. Highly crystalline lignocellulose requires harsher methods compared to the low crystalline ones. The potential of EFBs as a highly available cellulosic source has not been fully realized. The purpose of this study was to examine how the emerging method of microwave-assisted sequential acid/alkali pretreatment may be optimized to maximize hemicellulose and lignin removal, thereby increasing readily available cellulose for enzymatic saccharification and fermentation.

EXPERIMENTAL

Materials

Raw material

The EFB biomass was collected from the FELDA palm oil mill (Semenchu, Kota Tinggi, Johor, Malaysia). The EFB samples were washed with tap water to remove soil, dust, and other unwanted materials prior to air-drying under sunlight. The biomass was ground to 0.5–2 mm size using a disk mill (model FFC-15, Ah Koon Machinery & Trading Johor, Malaysia) and was sieved using a Restuch sieve shaker (AS 200 basis, Germany) into particle size less than 1.0 mm (mesh size < no. 18). Prior to analysis, the

powdered sample was dried in an oven at 60 °C for 24 h and stored in an airtight container at room temperature.

Chemical reagents

The following chemical reagents were obtained from Sigma Aldrich (Malaysia) and were of analytical grade: ethanol (C₂H₅OH), sulfuric acid (H₂SO₄), acetic acid (CH₃COOH), sodium hydroxide (NaOH), toluene (C₇H₈), sodium chlorite (NaClO₂) and sodium hypochlorite (NaClO) solution.

Methods

Conventional pretreatment

Dry EFB (20 g) with no physical treatment was soaked in 2.5 M of NaOH (20% w/v) for 2.0 h and then heated in an autoclave at 121 °C under a pressure of 0.12 MPa for 1.0 h. The alkali-treated EFB (EFB dilute acid) was washed with tap water followed by distilled water and filtered and dried at 90 °C overnight. The EFB was stored in tightly packed plastic bags for further analysis.

Dilute acid pretreatment

In sequential acid/microwave alkali-pretreatment, 20 g of dry EFB with no physical treatment was soaked in 8.0% (v/v) H₂SO₄ solution (20% w/v final concentration of EFB) and heated in an autoclave at 121 °C and 0.12 MPa for 1 h. The dilute acid-treated EFB (EFB acid) was then washed in the same volume of water, constantly mixed for 1 h and dried at 90 °C overnight. The autoclave filtrate was washed with tap water (4 x 1000 mL) followed by distilled water (4 x 500 mL). The second step was performed by adding 200 mL of fresh NaClO into the autoclave-treated biomass. The pH of solution was adjusted with the aid of a bench-top pH meter (Thermo-scientific Orion 2-star, Singapore) to acidic condition (pH 3.5) using CH₃COOH. The solution was then filtered, and acid-treated biomass was further washed using tap water (4 x 2000 mL). The dilute acid (DA)-treated oil palm biomass was then dried in oven at 70 °C and kept for subsequent analysis.

Microwave/alkali pretreatments of EFB

The dried EPB (10 g) acid after dilute acid pretreatment was then soaked in 2.5 N NaOH solutions at the ratio of 10:1 in a 250 mL glass vessel and irradiated in the microwave. A MAS-II microwave (SINEO Microwave, China) was used in the pretreatment. The condition set for microwave pretreatment is as follows: microwave power (700 - 1000 W), temperature (80-110 °C), and duration (60-90 min). The effect of factors such as microwave power, temperature, and time on the structure and composition of the EFB were analyzed. The microwave-treated EFB acid/alkali was soaked in water, continuously stirred and then washed with distilled water to remove NaOH on the surface of the biomass. The microwave-treated biomass was dried in an oven prior to subsequent analysis.

Determination of acid-insoluble lignin

Prior to quantification of acid-insoluble lignin (Klason lignin), raw EFB underwent thorough Soxhlet extraction to remove impurities. In Soxhlet extraction, ethanol and toluene were used in a ratio of 1:1 (volume) for 8 h, as described in TAPPI standard T204 (1997). Afterward, the samples were dried to a constant weight and the

insoluble lignin (Klason's lignin) was determined based on TAPPI standard method T222 om-02 (2002). First, 1 g of treated EFB was dissolved in 15 mL of 72% sulfuric acid solution and mixed gently with a glass rod for 2.0 h until the EFB was completely dissolved in acid solution. After 2 h, distilled water was added to the solution to reach a concentration of 4%, and the solution was heated in a water bath for 4 h. The residue was filtered, washed, and dried at 105 °C to constant weight. The amount of lignin residue in biomass was determined gravimetrically using Eq. 1:

$$\% \text{ Lignin} = \text{Wt. of lignin in grams} \times 100 / 1.0 \text{ g initial oven dried sample wt} \quad (1)$$

RESULTS AND DISCUSSION

The delignification achieved through the control condition that is the conventional pretreatment (CP) of EFB was 34.6%. In contrast, much higher delignification was achieved through a combination of closely examined factors in the microwave pretreatment dilute acid and microwave alkali (DAC + Mw-A), including microwave power, irradiation time and temperature. In the first step, dilute H₂SO₄ was used to remove 90% of hemicellulose and 10% lignin. In the second step, best delignification 71.9% was obtained with conditions of 900 W microwave power, 110 °C, and irradiation time of 80 min. In addition, microwave pretreatment (DAC + Mw-A) resulted in a lower amount of extractives as compared with conventional pretreatment (CP). Results for the delignification of oil palm lignocellulosic biomass EFB are shown in Table 1.

Table 1. Lignin Content of Oil Palm EFB Biomass (g/100 g Biomass)

Pretreatment Type	(g/100 g biomass)			
	Cellulose	Hemicellulose	Lignin	Extractives
Raw	41.8 ± 1.3	35.6 ± 1.6	18.8 ± 0.19	3.6 ± 0.20
CP	52.5 ± 1.4	29.4 ± 1.5	12.3 ± 0.30	2.8 ± 0.04
DAC + Mw-A	85.4 ± 1.3	3.5 ± 1.4	5.3 ± 0.10	1.7 ± 0.06
Data presented as the mean ± standard deviation of two set of replicates experiment				

Morphology of Pretreated EFP

The morphologies of non-treated EFB, dilute acid/alkali (DAC+Mw-A) pretreated EFB, and conventionally pretreated EFB are shown in Fig. 1. The volume of pretreated biomass decreased as compared with untreated biomass. Based on visual observations, the color of the DAC+Mw-A pretreated EFB is darker due to the maximum removal of hemicellulose. In contrast, the color of conventionally pretreated EFB is much paler and similar to the untreated sample. The surface of DAC+Mw-A pretreated EFB is more rough and irregularly ordered as compared with untreated EFB, which is quite rigid, clear, and well-ordered. The irregular structure of pretreated biomass increased the surface area and made it more accessible for enzymatic hydrolysis.

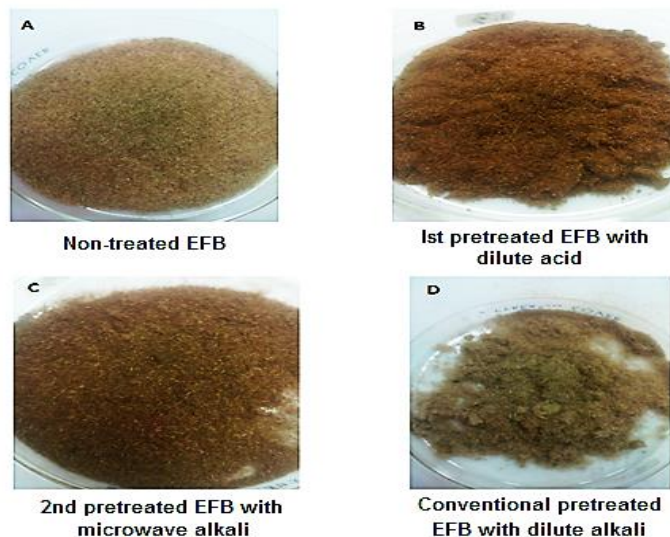


Fig. 1. Morphologies of the EFB samples: (A) non-treated EFB; (B) dilute acid-pretreated EFB; (C) sequential acid/alkali-pretreated EFB fiber; (D) conventional dilute alkali pretreated EFB

Effect of Microwave Power on Delignification

The effect of microwave power irradiation on oil palm EFB was studied. The microwave power was set in the range of 700 to 1000 W, while temperature and time were kept constant at 80 °C and 60 min, respectively. Microwave power has a direct effect on the structure disruption, as the amount of lignin decreases with an increase in microwave power. As shown in Fig. 2, the amount of lignin decreased directly as microwave power increased from 700 to 1000 W. The amount of lignin decreased from 13.4 to 9.3%, corresponding approximately to a delignification level of 30%. Microwave power from 700 to 900 W had a direct effect, as a higher amount (28.3%) of delignification was achieved. However, from 900 to 1000 W, only a negligible amount (1.87%) of lignin was removed.

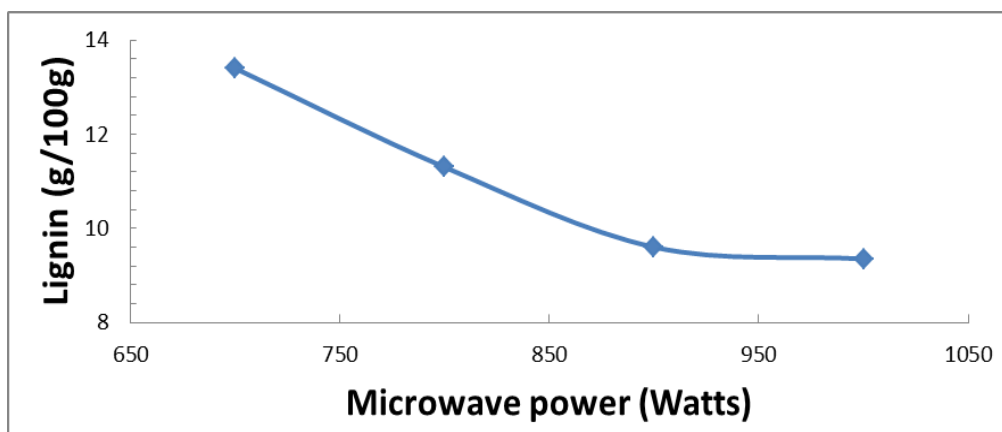


Fig. 2. Dependence of EFB delignification on microwave power

Similar studies on microwave power irradiation have been conducted with the pretreatment of corn stover and rice straw. For example, Pang *et al.* (2012) found that glucose and xylose yield increased with increasing microwave power irradiation, with

maximum sugar yield and delignification obtained at 540 W microwave power and 5 min irradiation time. Zhu *et al.* (2005) introduced a combination of microwave and alkali pretreatment for rice straw. The parameters set for the rice straw experiment were in the range of 300 to 700 W for 15 to 70 min, with microwave power increasing from 300 to 700 W at 15 min and microwave irradiation time increasing from 15 to 70 min at 300 W. Similar results were seen under both treatments, as the composition of cellulose (glucan) increased by 9.3%, while xylan and lignin decreased by 6.3% and 2.9%, respectively. These studies underscore how both a microwave operated at alkali conditions, or the combination of alkali and microwave pretreatment, yield the strongest results and can achieve high delignification.

Effect of Time on Delignification

As indicated by the research summarized above and from the results of the current study, short irradiation time with high microwave power or long irradiation time with low microwave power yield nearly identical results. In this study, the parameters were set for delignification at 60 to 90 min, with temperature and microwave power kept constant at 80 °C and 900 W respectively. The delignification achieved from 60 to 90 min was approximately 23.95%, while that achieved from 60 to 80 min was approximately 22.19%. However, from 80 to 90 min, lignin decreased by only 1.76%. The effect of irradiation time on delignification is shown in Fig. 3.

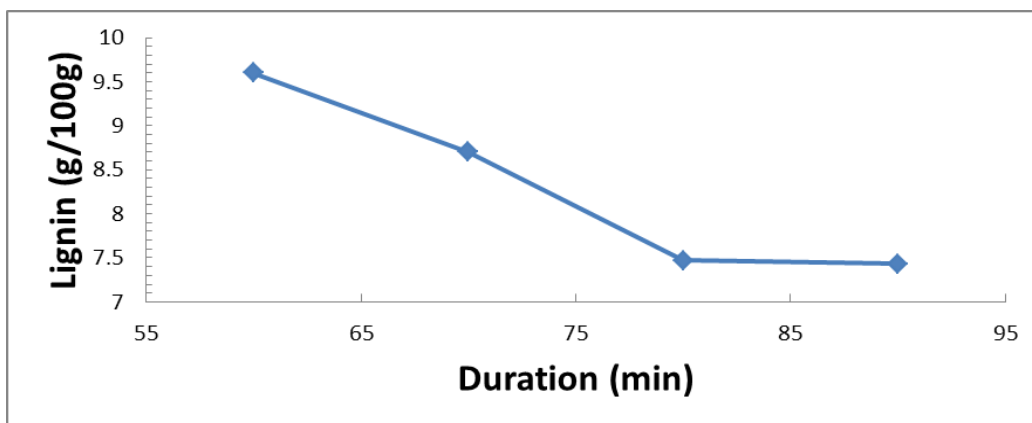


Fig. 3. Dependence of EFB delignification on time duration

The effect of time on delignification of biomass were studied by different researchers and obtained similar results. Beszédes *et al.* (2011) obtained the highest ethanol yield at 250 W microwave power for 10 min and a similar yield at 500 W microwave power for 5 min. However, such results are not always linear, as the first yield reached the maximum at 540 W with decreasing sugar yield thereafter. Presumably the phenomenon at work here is that a sudden increase in power increases temperature which break bonds between oxygen. This forms free radicals and thus more stable compounds (Zhu *et al.* 2005; Gong *et al.* 2010; Beszédes *et al.* 2011).

Effect of Temperature on Delignification

One of the advantages of using microwave heating is that it provides better results in less time as compared to conventional heating. In conventional heating, energy is applied from the bottom, while in a microwave treatment, the energy diffuses throughout

the entire sample. The high intensity of heating disrupts the crystalline structure of cellulose enhancing enzyme accessibility.

Energy transmitted to the sample during microwave irradiation excites dipole movement of the molecules, thereby increasing temperature. Increased irradiation time increases molecule collisions, which creates an alternative magnetic field and break hydrogen bond. However, after a certain duration, irradiation creates such high intramolecular energy that the hydrogen bonds can re-associate, thereby decreasing the effectiveness of pretreatment (Gong *et al.* 2010).

In this study, the effect of temperature on delignification was examined in the range of 80 to 110 °C while keeping microwave power and time constant at 900 W and 80 min, respectively. A substantial amount of delignification 24.4% was achieved from 60 to 100 °C, with a total of 29.3% achieved in the range of 60 to 110 °C. Thus, delignification counted for less than 20% of the total when temperature was raised from 100 to 110 °C. The effect of temperature on delignification is shown in Fig. 4.

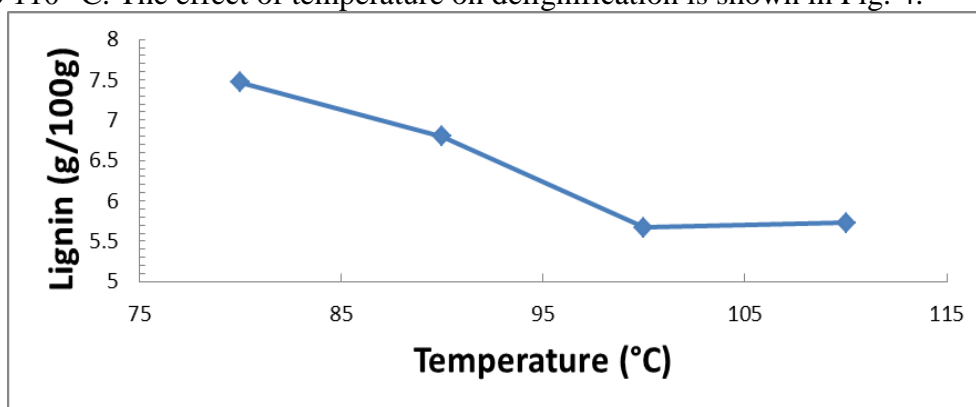


Fig. 4. Dependence of EFB delignification on temperature

CONCLUSIONS

1. The use of dilute acid and Mw-A is a rapid and effective pretreatment for EFB.
2. Different conditions for delignification from EFB were studied and the best yield was obtained at 900 W microwave power, 110 °C and 80 min.
3. Dilute acid in the first step removed 90% of hemicellulose and 10% of lignin.
4. In the second step, dilute alkali with microwave achieved 71.9% delignification.
5. Microwave-alkali successfully decreases the amount of hemicellulose and lignin and increase cellulosic content.
6. The recalcitrant structure of EFB is effectively disrupted through microwave irradiation.
7. Dilute acid renders EFB fibers rough, irregularly ordered, and porous, thereby increasing the surface area across which cellulose can be easily available for enzymatic saccharification.

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