COMPARISON OF PRETREATMENT STRATEGIES FOR CONVERSION OF COCONUT HUSK FIBER TO FERMENTABLE SUGARS

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In the present study, coconut husk was employed as biomass feedstock for production of bioethanol, due to its abundance in Malaysia. Due to the complex structures of coconut husk, a pretreatment process is crucial in extracting fermentable sugars from the embedded cellulose matrix for subsequent ethanol fermentation process. The ground coconut husk was subjected to three different pretreatment processes inclusive of thermal, chemical, and microwave-assisted-alkaline techniques, prior to enzymatic hydrolysis and fermentation process. The composition profile of coconut husk was significantly altered upon the microwave-assisted-alkaline treatment as compared to the untreated sample, with the cellulose content increasing from 18-21% to 38-39% while lignin content decreased from 46-53% to 31-33%. Among the pretreatment methods applied, enzymatic hydrolysis of coconut husk pretreated by microwave-assisted-alkaline method recorded the highest yield of fermentable sugars, 0.279 g sugar/g substrate. SEM imaging showed the obvious and significant disruption of coconut husks' structure after microwave-assisted-alkaline pretreatment. In conclusion, by employing suitable pretreatment technique in treating the lignocellulosic materials of coconut husk, the extracted fermentable sugar is a potential substrate for bioethanol production.

Keywords: Lignocellulosic; Microwave-assisted-alkaline pretreatment; Coconut husks; Fermentable sugar

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INTRODUCTION

In recent years, bio-ethanol has been considered a better choice than conventional fuels, as it reduces the dependence on reserves of crude oil. Bio-ethanol also promises cleaner combustion, which may lead to a healthier environment because it is carbon 'neutral' and essentially free from sulfur and aromatics (Gupta et al. 2008). Today, bio-ethanol is one of the most dominant biofuel and its global production has increased sharply since year 2000. Generally, current production of bio-ethanol comes from both sugar and starch-based materials such as sugarcane and grains (Dermirbas 2009). However, considering the growing demand for human food, lignocellulosic materials has arisen as a more suitable feedstock for bio-ethanol production compared to the other two groups of raw material.

Coconuts are abundantly growing in coastal areas of all tropical countries, and its production from the top ten coconut producing countries had reached 54,716,444 tonnes by the year 2008 (FAO statistics 2008). The coconut husks are routinely disposed of after the coconut water is sold. This makes coconut husk a cheap and potential substrate that could be used for bio-ethanol production due to the presence of relatively high levels of cellulose and hemicelluloses in it (van Dam et al. 2004). These two components, after pretreatment, are more accessible to enzymatic hydrolysis and hence are a potential source of fermentable sugar for bio-ethanol production process.

Generally, pretreatment methods can be categorized into physical, chemical, and biological pretreatment (Wyman 1996). Physical pretreatment involves some mechanical actions, where it disrupts the cell wall components of the lignocellulosic substances. The examples of physical pre-treatment are milling, irradiation, and heat or steam treatment. Chemical pretreatment, on the other hand, involves the application of chemical solutions to dissolve the lignins, celluloses, and hemicelluloses found in the lignocellulosic substances. The examples are alkaline treatment, acid treatment, ozonolysis treatment, and wet oxidation. Biological pretreatment usually involves the use of microorganisms to degrade the lignin and hemicelluloses, however, this method is not effective as the degradation process is significantly slow as compared to other methods (Taherzadeh and Karimi 2008). Hence, pretreatment and hydrolysis steps are necessary in order to extract and obtain the fermentable sugars for the subsequent bioethanol fermentation process. The aims of the present study were to investigate and identify the most effective pretreatment method that could increase the final yield of fermentable sugars from coconut husks.

EXPERIMENTAL

Materials

Collection and processing of coconut husks

A commonly available agricultural waste, coconut husk, was collected from a coconut plantation site. This waste was then cut into small pieces (ca. 5 cm) and processed to remove dirt or debris.

Methods

Pretreatment of wastes

Three pretreatment methods, i.e. thermal, chemical, and microwave-assisted-alkaline (MAA), were conducted to evaluate their effects on the structures of coconut husks. The experiment was carried out by employing two particle sizes of coconut husks (300-600 μm^2 and 850-1500 μm^2) with 10% moisture content (NREL 2009). Both sizes of the ground coconut husks were subjected to the three pretreatment methods.

In thermal pretreatment, the ground coconut husks were autoclaved at 121°C and 1.034 bar for 15 minutes. The heat-treated coconut husks were then dried in an oven to remove excessive moisture and kept for further use.

Chemical pretreatments involved either a solution of acid (1% v/v H_2SO_4) or alkali (5% w/v NaOH) were subjected to the ground coconut husks, with 50:1 of liquid to solid ratio. The mixture was incubated in a shaking incubator at 40°C and 150 rpm for 24 h. Then, the treated coconut husks were filtered and washed with distilled water until the pH was neutral. Subsequently, the cleaned coconut husks were then dried in an oven to remove excessive moisture and kept for further use.

In MAA pretreatment, the biomass (6 g of dry basis) was immersed in a diluted NaOH solution (5% w/v), and this slurry was then exposed to microwave radiation in a domestic microwave oven (Sharp, R-218(S)) at 2450 MHz for 20 minutes. After 20 minutes, the mixture was rinsed with distilled water to remove excess alkaline solution. The residue were then dried in an oven and kept for subsequent use.

Sacchrification of treated waste on the production of fermentable sugars

Two saccharifying commercial enzymes, Pectinase (Pectinex ® ULTRA SP-L, Novozymes, Denmark), and cellulase (Celluclast ® 1.5 L, Novozymes, Denmark) were used to hydrolyse the treated coconut husks. In a 250 mL of Erlenmeyer flask, cellulase and pectinase (0.5% v/v each) were added to 100 mL of sterile distilled water containing 1% (w/v) of coconut husks. The saccharification process was carried out at 35°C at 150 rpm for 5 days. Samples were withdrawn every 12 h for analysis of sugar concentration. The content of reducing sugars was determined using the 3,5-dinitrosalicylic acid method at 540 nm (Miller 1959).

Feedstock characterisation: cellulose, hemicelluloses and lignin of coconut husks

The characterization of treated and untreated coconut husks was conducted according to the acid detergent fiber, neutral detergent fiber, and acid detergent lignin (ADF-NDF-ADL) method (Goering and van Soest 1970). Hemicellulose and cellulose were calculated based on Eqs. 1 and 2.

$$\text{Hemicellulose (\%)} = \text{NDF (\%)} - \text{ADF (\%)} \qquad (1)$$

$$\text{Cellulose (\%)} = \text{NDF(\%)} - \text{Hemicellulose (\%)} - \text{Lignin (\%)} \qquad (2)$$

Scanning electron microscopy (SEM) analysis (JEOL, model JSM-6400 SEM) was conducted in Universiti Putra Malaysia, Malaysia in order to view and compare the changes of physical structure of treated and untreated coconut husk. The specimen of SEM was cut into a number of 1 cm^2 tissues.

The tissue was put into separate vials and fixed in 4% buffered glutaraldehyde for 24 h at 4^oC. The specimens were then washed with 0.1 M sodium cacodylate buffer for 3 changes of 10 min each. The specimens were post-fixed in 1% buffered osmium tetroxide for 2 h at 4^oC. Following that, the washing of specimens with 0.1 M sodium cacodylate was repeated. Then, dehydration was conducted to remove unbound water with a series of ethanol ranging from 10% to 100%. The specimen after dehydration process was then transferred into specimen basket and was put into critical point dryer for about 45 min. Finally, the samples were gold-coated in a sputter coater and were ready for SEM viewing.

RESULTS AND DISCUSSION

As illustrated in Table 1, the MAA treatment increased the cellulose content of coconut husks to 38-39%, while it reduced the lignin content from 46-53% to 31-33%, in comparison to cellulose content of physically treated coconut husk (18-21%). Alkaline pretreatment is believed to cause swelling of the lignocellulosic structure, leading to an increase in internal surface area, separation of structural linkage between lignin and carbohydrates, and the disruption of lignin structure (Ong et al. 2010; Kashaninejad and Tabil 2011). As described in the literature, microwave treatment produces a rapid volumetric heating throughout the material, resulting in alteration of physio-chemical characteristics of the material, which enables and accelerates the breaking down of the lignin-hemicellulose complex and increases the exposure of cellulose surface to cellulase (Ma et al. 2009; Jackowiak et al. 2011). In addition, Gabhane et al. (2011) reported that microwave treatment has better efficacy (more than 10% improvement in sugar yield) on garden biomass than autoclave and hot plate treatment techniques. Hence, the combination of microwave and alkaline treatments techniques could improve the hydrolysis process by accelerating the main reaction of alkaline-pretreatment-delignification. Consequently, lignin content of the MAA pretreated coconut husk had been decreased significantly as compared to coconut husks treated by other treatment methods. The removal of lignin content helps in exposing more cellulose fibers to the saccharifying enzymes.

Table 1. Cellulose, Hemicellulose and Lignin Contents of Pretreated Coconut Husks

Pretreatment	Particle size (μm^2)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Untreated	300 - 600	21.26 ± 1.51^a	17.33 ± 0.74^a	46.36 ± 0.57^a
	850 -1500	18.19 ± 2.81^b	11.34 ± 1.34^b	53.08 ± 2.37^a
Thermal	300 - 600	19.21 ± 1.39^c	13.92 ± 0.37^c	50.90 ± 0.82^b
	850 - 1500	23.36 ± 1.48^c	14.31 ± 0.93^c	51.71 ± 1.39^c
Acid	300 - 600	16.98 ± 4.19^d	22.36 ± 2.99^d	48.65 ± 0.08^d
	850 - 1500	25.60 ± 1.75^d	13.20 ± 0.42^e	51.50 ± 0.68^e
Alkaline	300 - 600	36.87 ± 0.88^e	22.63 ± 0.25^f	36.76 ± 0.86^f
	850 - 1500	33.74 ± 0.77^e	24.23 ± 1.10^f	37.59 ± 0.49^g
MAA	300 – 600	38.93 ± 1.94^f	25.04 ± 0.93^g	32.98 ± 1.62^h
	850 – 1500	39.98 ± 1.45^f	25.25 ± 0.79^g	31.79 ± 1.07^i

$^{a-i}$ mean values in the same column not followed by the same letter are significantly different ($P < 0.05$).

As shown in Fig. 1, the MAA pretreatment gave a significant boost to the reducing sugar yield in the hydrolysis step (0.56-0.58 g/L to 2.16-2.79 g/L) as compared to control and other pretreatment techniques. As shown by previous work, the MAA technique is an effective pretreatment method in treating lignocellulosic materials of rice straw (Zhu et al. 2005), wheat straw (Zhu 2006), switchgrass (Hu and Wen 2008), and sugarcane baggase (Binod et al. 2012). Microwave pretreatment led to much higher xylose content from switchgrass as compared with conventional heating; however, individual treatment by using microwave alone cannot totally break down the recalcitrant structures of lignocellulose, as it only helps to facilitate the treatment process (Hu and Wen 2008). In addition, Li et al. (2011) also stated that pretreatment by microwave helps to assist the decrease of the cellulose crystallisation as compared with conventional heating. Therefore, the increased production of reducing sugars is predominantly attributed to the greater exposure of cellulose surface area of the microwave-assisted-alkaline treated lignocellulosic biomass (Kumar et al. 2009).

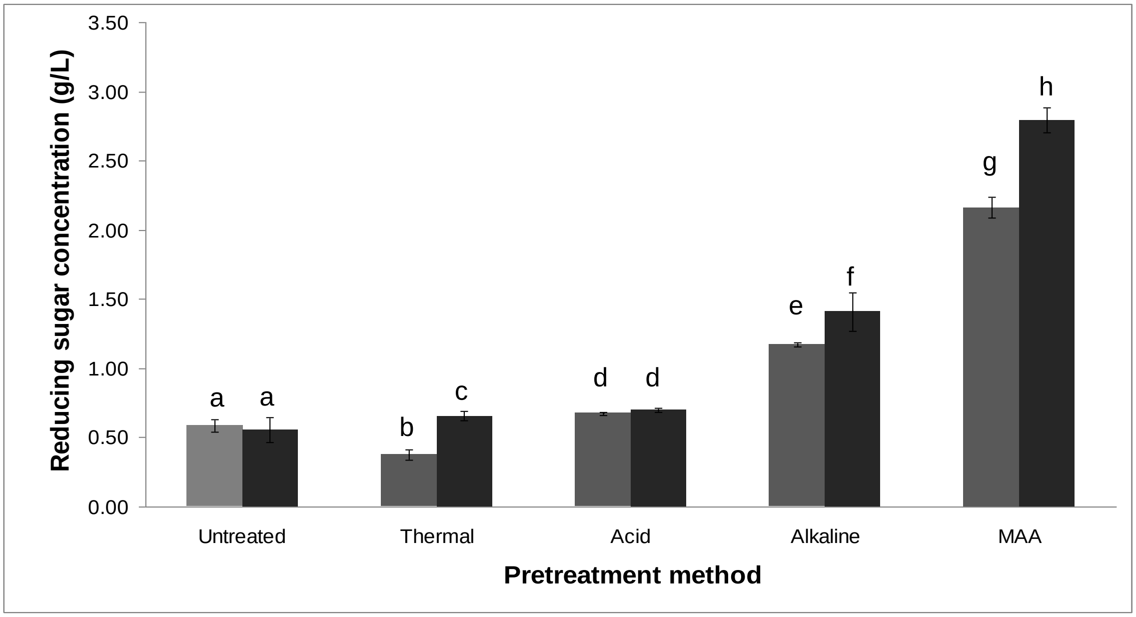

Figure 1. Maximum level of reducing sugar produced from the pre-treated coconut husks. Symbols:(▨), 300-600μm^2; (■), 850-1500μm^2. Error bars indicate the mean ± standard deviation of three experiments. $^{a-h}$ mean values in the graph not followed by the same letter are significantly different ($P<0.05$).

Scanning electron microscope (SEM) images of the untreated and pretreated coconut husks are shown in Fig. 2. The content and structure of coconut husks had changed noticeably through the alkaline treatment as compared to untreated, thermal, and acid treatment (Table 1 and Fig. 1).

The initially smooth structure of untreated coconut husks (Fig. 2A) had been damaged to a sufficient extent that surface of alkaline-treated coconut husks had become roughened and loosened (Fig. 2D). MAA pretreatment had successfully altered the initially organized morphology of coconut husks into rugged and unorganized structures (Fig. 2E). Moreover, when the alkaline pretreatment was combined with the microwave irradiation, the yield of sugar after hydrolysis was markedly increased approximately 1-fold (Fig. 1).

Hu and Wen (2008) noticed that the lignocellulosic material became “thinner and striated” under SEM analysis after the materials had been presoaked in alkali and followed by microwave irradiation treatment. It is believed that the lignin was degraded and hence increased the exposure of cellulose and hemicellulose in the lignocellulosic materials to hydrolysing enzymes such as cellulase (Hu and Wen 2008).

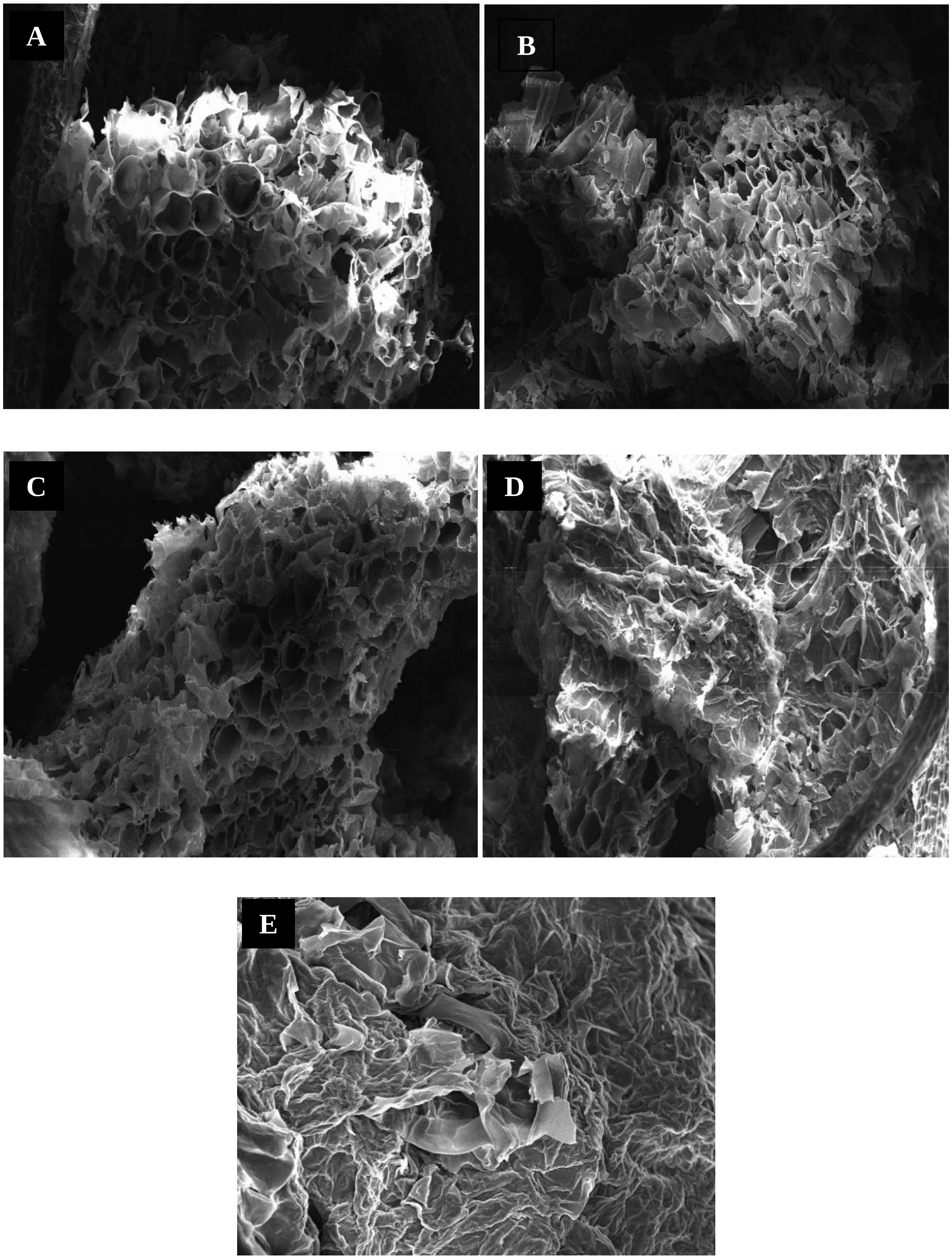

Figure 2. SEM images of coconut husks. (A) Control, (B) Thermal, (C) Acid, (D) Alkaline, and (E) Microwave-assisted-alkaline (magnification = 100×)

CONCLUSIONS

The general goal of pretreatment process is to alter or remove structural and compositional impediments in lignocelluloses in order to improve the rate of enzymatic hydrolysis and increase yields of fermentable sugars from celluloses. In the present study, the most effective pretreatment method in releasing the highest concentration of reducing sugars from coconut husks after enzymatic hydrolysis was the microwave-assisted-alkaline pretreatment. By using MAA pretreated coconut husks with sizes in between 850 μm^2 and 1.5 mm^2, the maximum concentration and productivity of reducing sugar reached was 2.79 g/L and 0.058 g/L.h, respectively.

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