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The Potential of Coconut Husk Utilization for Bioethanol Production

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ABSTRACT

Coconut (*Cocos nucifera*) husk, a lignocellulosic residue, contained 39.31% alphacellulose, 16.15% hemicellulose, 29.79% lignin and 28.48% extractives. In this study, the possibility of using coconut husk as a substrate for bioethanol production was investigated. The coconut husk was treated with 20, 25 and 30% sodium hydroxide solution at 100°C for 2-3 h under pressure to obtain the coconut husk cellulose for ethanol conversion. The commercial enzymes, Celluclast 1.5L and Novozyme 188, were used for cellulose hydrolysis, and yeast (*Saccharomyces cerevisiae*) was used for ethanol fermentation. The simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) processes were compared for ethanol conversion efficiency. The results showed high ethanol productivity of the coconut husk cellulose from the SHF and SSF processes at 21.21 and 20.67% (based on pulp weight), respectively.

Keywords: ethanol, coconut husk, production, separate hydrolysis and fermentation, simultaneous saccharification and fermentation

INTRODUCTION

The energy crisis is one of the most serious problems facing the sustainability of human civilization. Furthermore, demand for petroleum-derived fuels has increased substantially over the past few decades (Goh *et al*., 2009). As a result, the search for a long-term solution for a reliable and infinite source of energy supply in the future has been a tremendous challenge. Lignocellulose biomass, which comprises mainly cellulose, hemicellulose and lignin, has been considered as a second-generation feedstock for bio-ethanol production. Thailand is one country that creates a substantial amount of lignocellulose agricultural waste, which includes from coconuts. Usually, 5.6 million tonnes of coconut and 1.1 million tonnes of coconut husk are produced annually (Mr. Wuttinunt Kongtud, pers. comm.). Coconut (*Cocos nucifera*) husk is attractive due to its high proportions of well-defined polymeric structures of cellulose, hemicellulose and lignin with 28, 38 and 32.8 %, respectively (Pollard *et al*., 1992). Efforts have already been made to create value from these components as a precursor for the preparation of fibers that can be incorporated in cementitious matrices (Bilba *et al*., 2007) and as potential adsorbents in wastewater purification (Pollard *et al*., 1992; Hasany and Ahmad, 2006; Anirudhan

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et al., 2008; Namasivayam and Sangeetha, 2008). However, coconut-derived cellulose has been considered as one of the renewable sources for the production of environmental friendly bio-ethanol.

The objectives of this study were: 1) to determine the pretreatment of coconut husk for the preparation of coconut husk pulp for ethanol fermentation; and 2) to compare the efficiency of cellulose conversion into ethanol by the separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) processes.

MATERIALS AND METHODS

Preparation of coconut husk

Young coconut husks were used in the study. After drying, they were cut into pieces approximately 25 × 25 × 5 mm by a laboratory chipper. The experiments used a factorial completely randomized design with the two factors being NaOH concentration (20, 25 and 30% based on raw material dry weight) and boiling time (2 and 3 h), as shown in Table 1. All treatments were performed at 100°C with two replications. Then, the treated fiber was subjected to the 25% (based on fiber dry weight) NaOH solution at 170°C for 3 h under pressure to obtain coconut husk pulp. Excess chemical was removed by thorough washing with water, the pulp was oven-dried and used as substrate for further ethanol fermentation.

Preparation of inoculum

The fermentation yeast, *Saccharomyces cerevisiae*, was initiated in YPD slant (10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose, 15 g/L agar) at room temperature (25-30°C) for 2 d. To prepare the inoculum, yeast was subcultured into fresh media (50 g/L glucose, 0.5 g/L $(NH_4)_2HPO_4$, 0.025 g/L $MgSO_4 \cdot 7H_2O$, 12 g/L NaH_2PO_4, 1 g/L yeast extract) for 24 h at 200 rpm on a rotary shaker at 30°C. Then, the yeast cells were harvested by centrifugation at × 8000 *g* for 15 min and then washed with sterile water before being used for ethanol fermentation.

Ethanol production by SHF process

The pretreated young coconut husk pulp was subjected to an enzymatic hydrolysis to obtain the monomeric glucose. The combination of two commercial enzymes, Celluclast 1.5 L and Novozyme 188, at activities of 15 FPU and 15 IU per 1 g substrate, respectively, were applied. Five percent of substrate (w/v) was used. The hydrolysis reaction was performed at pH 4.8 and 50°C for 72 h with 140 rpm shaking. Subsequently, the hydrolyzed glucose solution was adjusted to pH 5.5 by the addition of 20 % $Ca(OH)_2$ solution. Then, this solution was autoclaved at 121°C for 20 min. The ethanol fermentation process was performed in a total volume of 125 mL using 5 g/L *S. cerevisiae* cells, 50 g/L glucose, 0.5 g/L $(NH_4)HPO_4$, 0.025 g/L $MgSO_4.7H_2O$, 1.2 g/L NaH_2PO_4 and 1 g/L yeast extract at 30°C for 72

Table 1 Pulp yield obtained with various NaOH concentrations and boiling times.

Run	NaOH (%)	Time(h)	Fiber yield (%)	Solid residue (%)	Fiber characteristic
1	20	2	46.06[a]	5.04[a]	Poor fiber separation
2	20	3	45.98[a]	5.90[a]	Poor fiber separation
3	25	2	40.98[b]	6.68[a]	Good fiber separation
4	25	3	40.50[b]	5.96[a]	Good fiber separation
5	30	2	39.46[b]	5.90[a]	Good fiber separation
6	30	3	38.26[b]	5.08[a]	Good fiber separation
SD			1.414		

Means in the same row with different letters are significantly different at the 95% level ($P < 0.05$).

h. The samples were collected at time intervals for the analysis of remaining glucose and produced ethanol.

Ethanol production by SSF process

Each pretreated young coconut husk sample was simultaneously hydrolyzed and fermented in a total volume of 125 mL. First, the coconut husk cellulose solution at 5 g/L was prepared, the pH was adjusted to 5.5 and the solution was autoclaved at 121°C for 20 min. Then, 15 FPU Celluclast1.5L and 15 IU Novozyme188 per 1 g substrate were added. The fermentation process was performed under the same conditions as for the SHF process, except that the SSF process was carried out at 37°C for 72 h. The samples were collected at time intervals for the analysis of remaining glucose and produced ethanol.

Analytical methods

A part of the coconut husk sample was ground with a Wiley mill (Kinematica AG Co. Ltd., Tokyo, Japan), then passed through a 420-μm sieve prior to chemical analyses. The contents of lignin, alphacellulose and hemicellulose were determined using the Technical Association Pulp and Paper Industry method (TAPPI, 1996). The glucose and xylose contents were quantitatively determined by high performance liquid chromatography (HPLC) connecting to a refractive index (RI) detector. An AMINEX HPX87P carbohydrate analysis column (Bio-Rad, Hercules, CA) was operated at 85°C using deionized water as a mobile phase at 0.6 mL/min. Ethanol was measured by gas chromatography (GC) using an HP5890 Series II apparatus equipped with an Agilent 6890 Series injector.

RESULTS AND DISCUSSION

The results showed that the coconut husk contained 39.31% alphacellulose, 16.15% hemicellulose, 29.79% lignin and 28.48% extractives. The content of alphacellulose was as high as that in an empty fruit bunch of oil palm (47.83%), indicating the possibility of high ethanol conversion from coconut husk. High lignin and extractive contents in the coconut husk indicated the need for pretreatment of this raw material prior to ethanol fermentation (Prasad *et al*., 1983). The conversion of lignocellulosic biomass to ethanol usually employs three major steps: 1) pretreatment, to breakdown the lignin and open the cellulose structure; 2) hydrolysis, with a combination of enzymes to convert the cellulose to glucose; and 3) microbial fermentation of the glucose to ethanol. Existing pretreatment methods have largely been developed. Chemical treatment is widely used since this method efficiently removes hemicellulose and lignin from cellulose; however, optimization of the treatment conditions is needed (Brígida *et al*., 2009). In the present study, the treatment with NaOH solution was able to remove a little of the lignin (yielding 28.53% lignin) but a lot of the extractives (yielding 3.06% extractives), which resulted in higher alphacellulose (48.90%) and hemicellulose (22.04%). Table 1 presents the fiber yield and solid residues obtained from the treatments using various NaOH concentrations and heating times at 100°C. The test for significant difference was set at the 95 % level ($P < 0.05$). There was no significant difference among the amounts of solid residue from all treatment conditions. No significant difference in the fiber yield between the conditions with 25 and 30% NaOH was observed, whereas the treatment with 20% NaOH gave significantly higher fiber yield. However, the resulting fiber from the 20% NaOH treatment was not well separated based on visual observation. The poor separation of the cellulose fiber limited cellulose hydrolysis by the enzyme. The treatment with 25% NaOH at 100°C for 3 h was then selected for the preparation of young coconut husk fiber. The treated coconut husk fiber was also further treated with 25% (based on fiber dry weight) NaOH solution at 170°C for 3 h under

pressure to obtain coconut husk pulp for ethanol production by SSF and SHF.

For the SHF process, the pulp was first hydrolyzed into single glucose molecules in one reactor and then, in the second step, the reaction mixture was transferred to another reactor for yeast fermentation to ethanol. The amount of glucose obtained from enzymatic hydrolysis of coconut husk pulp is shown in Figure 1. The glucose concentration in the hydrolysate varied directly with the reaction time, increasing as the reaction time lengthened. The amount of released glucose increased dramatically during the first 24 h and then became constant. After 72 h, the glucose concentration reached 22.80 g/L, which was equivalent to 45.59% based on the initial pulp weight. This indicated that more than 50% of the coconut husk pulp was not hydrolyzed and still remained as a polymer of glucose. Thus, further optimization was essential in order to improve the efficiency of cellulose hydrolysis. Figure 2 documents the ethanol fermentation of the

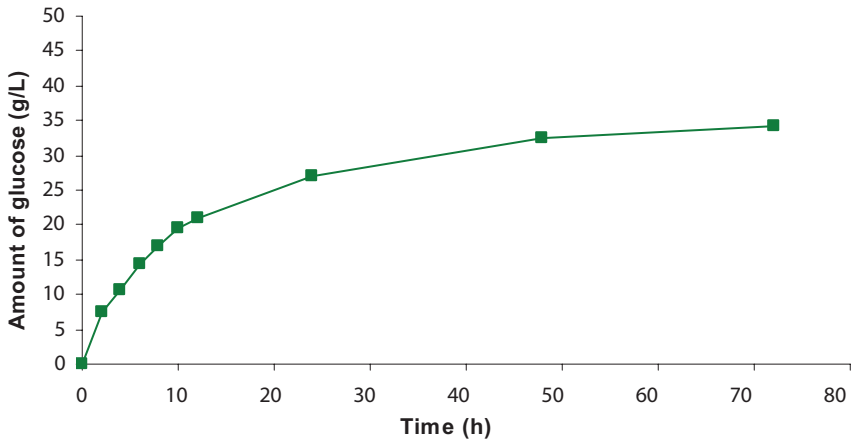

Figure 1 Hydrolysis of young coconut husk pulp in the SHF process with 15 FPU Celluclast1.5L and 15 IU Novozyme188 per 1 g substrate at pH 4.8, 50°C and 140 rpm shaking for 72 h.

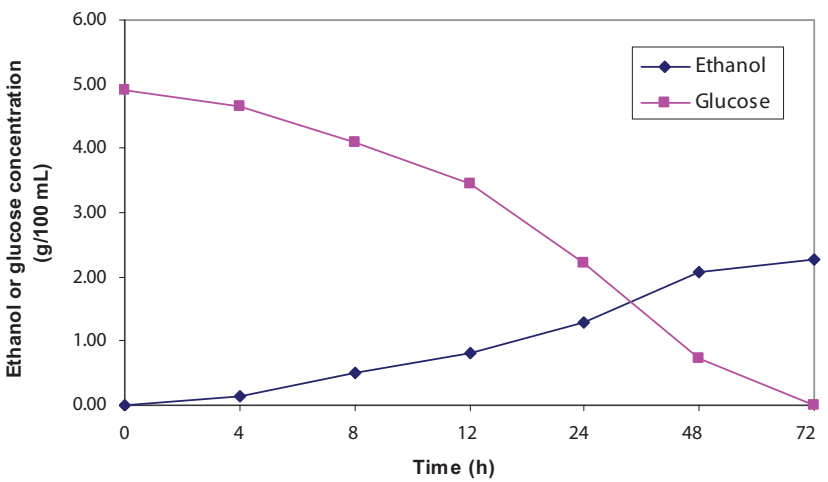

Figure 2 Ethanol fermentation of hydrolyzed glucose by *S. cerevisiae* in the SHF process at 30°C for 72 h.

hydrolyzed glucose at various times. The amount of ethanol produced increased constantly and reached 2.28% (equivalent to 91.04% of the theoretical yield) after 72 h. This result was consistent with the gradual reduction of the glucose substrate in the reaction.

For the SSF process, the coconut husk pulp was enzymatically hydrolyzed and the yeast was fermented simultaneously in the same reactor. Thus, the efficiency was heavily dependent on both hydrolysis and fermentation. Figure 3 shows the increase in ethanol produced as fermentation increased and the production reached its maximum level at 1.03% (w/v) which was equivalent to 20.67% based on the initial pulp weight. The glucose content was also determined in order to investigate the enzymatic hydrolysis of cellulose. However, no glucose was detected in the reaction since it was able to be immediately converted into ethanol right after hydrolysis.

CONCLUSION

The possibility of ethanol production from young coconut husk was investigated. The amounts of ethanol produced by the SHF and SSF processes were compared. There was a similar ethanol yield from the SHF (21.21% based on pulp weight) and SSF (20.67% based on pulp weight) processes. The ethanol yields were approximately 85% of the theoretical ethanol yield. Overall, the results showed that ethanol production from agricultural residues such as coconut husk was promising. However, optimization of the process at each stage should be further studied in order to develop the most suitable conversion technology.

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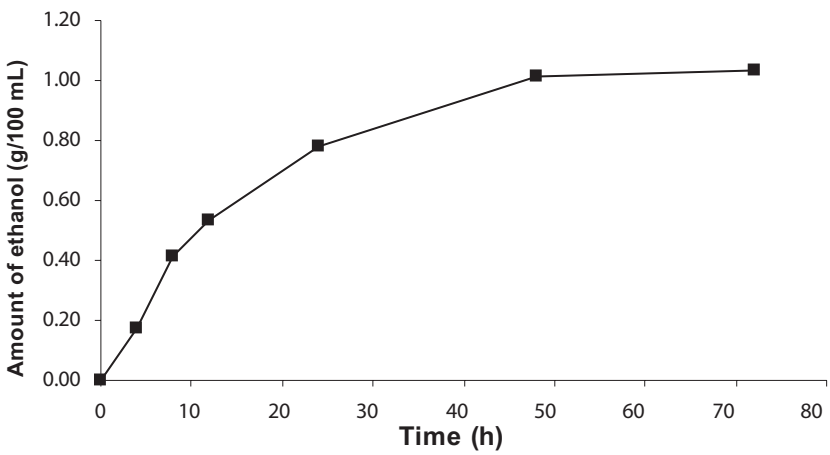

Figure 3 Ethanol produced by SSF process with young coconut husk pulp. The reaction contained 15 FPU Celluclast 1.5 L and 15 IU Novozyme188 per 1 g substrate and was carried out at 37°C for 72 h.

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