Saccharification of Pretreated Oil Palm Empty Fruit Bunch Fiber Using Cellulase of *Chaetomium globosum*

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The effectiveness of different chemical and physical pretreatments to alter cellulose structure and to reduce hemicellullose and lignin contents in oil palm empty fruit bunch (OPEFB) fibers for subsequent use as substrate for enzymatic saccharification was studied. The saccharification was carried out using concentrated cellulase preparation from culture broth of *Chaetomium globosum* strain 414 containing 10 U/mL FPase, 285 U/mL CMCase, and 60 U/mL β-glucosidase. The use of 0.5% NaOH to treat OPEFB by soaking at 30 °C for 4 h gave the highest rate and degree of hydrolysis followed by 0.5% HNO_3, HCl, EDA, and EDTA. Autoclaving the chemically treated OPEFB fiber at 121 °C for 5 min improved the hydrolysis by 2-fold. The improvement in hydrolysis was related to a decrease in the hemicellulose and lignin contents and an increase in the cellulose content. The qualitative hydrolysis yield for autoclaved OPEFB fiber treated with 2% NaOH was 85.9%. During saccharification of OPEFB using cellulase of *C. globosum*, the amount of glucose produced was higher while the amount of cellobiose produced was lower than those obtained with commercial cellulase of *Trichoderma viride*.

Keywords: *Cellulase; endoglucanase; cellobiohydrolase; β-glucosidase; Chaetomium globosum; cellulosic material; oil palm empty fruit bunch*

INTRODUCTION

Over the past decades, lignocellulosic materials such as agricultural residues have been used as substrates for bioconversion into sugars that can be fermented to feed additives, single cell protein, fuels, and chemicals. In the industrial processing of oil palm fruit into oil, a large amount of oil palm empty fruit bunch (OPEFB) is generated. Having little commercial value, they become a disposal problem due to bulk density, thus occupying large storage volume and usually employed as fuel in the factory (Ma et al., 1993). As a lignocellulosic material, OPEFB represent a renewable and low-cost raw material resource for production of fermentable sugars or for transformation into chemicals and other high value-added products.

The most critical properties limiting the enzymatic saccharification of insoluble cellulose are (i) decreased reaction rate due to decreased substrate susceptibility to enzymes (Lee and Fan, 1983; Sinitsyn et al., 1991), (ii) end product inhibition (Ramos et al., 1993), (iii) reversible inactivation of enzymes adsorbed on cellulose surfaces (Gusakov et al., 1985), and (iv) their inactivation on lignin fragments (Ooshima et al., 1990). Polysaccharides in lignocellulosic materials are associated with lignin, which makes their hydrolysis difficult. The low susceptibility of the lignocellulosic materials to hydrolysis can be improved by physical, chemical, and biological pretreatments, especially in removing the lignin content to make the resultant polysaccharide fractions accessible to enzyme actions.

The most frequently reported source of cellulolytic enzymes is the cellulase of *Trichoderma reesei* (Persson et al., 1991). However, cellulase from this source has a relatively low specific activity, low thermostability, and high sensitivity to product inhibition (Mandels, 1985; Klyosov, 1988). In addition, the ratio of β-glucosidase to FPase for cellulase of *T. reesei* is rather low (Ryu and Mandels, 1980). A significant amount of β-glucosidase in cellulase preparation is required to avoid enzyme inhibition (Woodward and Wiseman, 1982; Ghani and Rickard, 1990). During the saccharification process, cellobiohydrolase acts synergistically with endoglucanase to hydrolyze crystalline cellulose. During the reaction, cellobiose is produced and inhibits both types of cellulases. Hence, a significant amount of β-glucosidase is required to hydrolyze cellobiose to relieve the inhibition. Recently, cellulase with high amount of β-glucosidase (ratio of β-glucosidase to FPase of 7) was obtained from *Chaetomium globosum* strain 414 (Umikalsom et al., 1997). Cellulase of this fungus might be used to overcome the problems related to the economic viability of the enzymatic saccharification process.

This study was aimed at investigating the effect of different chemical and physical treatments on OPEFB fibers for subsequent use in saccharification process. Cellulase of *Chaetomium globosum* strain 414, which has a high ratio of β-glucosidase to FPase, was used in all saccharification processes. The yield of reducing sugar and overall productivity obtained from the saccharification processes using OPEFB fibers treated with different chemical and physical methods were compared.

MATERIALS AND METHODS

Cellulase Enzyme Preparation. Cellulase enzyme produced by the fungus *Chaetomium globosum* strain 414 was used in this study. The basal medium described by Mandels and Weber (1969) with the addition of pretreated OPEFB fibers (10 g/L) and peptone (6 g/L) as carbon and nitrogen sources was used for cellulase production. Batch production

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Table 1. Chemical Composition of Untreated and Treated 2 mm OPEFB Fibers[a]

treatment	chemical composition (%)				
	cellulose	hemicellulose	lignin	ash	others
untreated	50.4 ± 1.2	21.9 ± 1.4	10.0 ± 1.7	0.5 ± 0.02	17.2 ± 4.32
chemical treatment without autoclaving					
0.5% NaOH	50.7 ± 2.8	20.7 ± 0.9	10.0 ± 0.5	0.8 ± 0.03	17.8 ± 4.23
2.0% NaOH	51.2 ± 1.8	19.0 ± 1.4	8.2 ± 0.2	0.9 ± 0.02	20.7 ± 3.52
0.5% HNO_3	50.2 ± 2.0	20.2 ± 1.0	8.5 ± 0.6	1.2 ± 0.1	19.9 ± 3.70
0.5% HCl	50.1 ± 1.7	20.3 ± 1.1	8.9 ± 0.3	0.7 ± 0.09	20.0 ± 3.19
0.5% EDA	50.1 ± 1.1	20.3 ± 1.0	8.8 ± 0.8	0.6 ± 0.03	19.2 ± 2.93
0.5% EDTA	50.0 ± 1.0	20.5 ± 1.2	9.3 ± 0.4	0.5 ± 0.02	19.7 ± 2.62
chemical treatments with autoclaving					
autoclaved without chemical treatment	49.7 ± 2.1	21.6 ± 1.4	11.7 ± 0.6	0.5 ± 0.01	16.5 ± 4.11
0.5% NaOH	52.9 ± 1.5	18.6 ± 1.5	8.3 ± 0.7	0.2 ± 0.01	20.0 ± 3.71
2.0% NaOH	59.5 ± 1.6	4.4 ± 1.2	3.7 ± 0.9	0.4 ± 0.01	32.0 ± 3.71
0.5% HNO_3	62.9 ± 0.9	4.6 ± 0.2	4.5 ± 0.4	0.7 ± 0.04	27.3 ± 1.54
0.5% HCl	55.5 ± 1.9	8.2 ± 0.6	6.3 ± 0.4	0.6 ± 0.02	29.4 ± 2.92
0.5% EDA	51.2 ± 1.8	9.4 ± 0.9	8.9 ± 0.8	0.3 ± 0.01	30.2 ± 3.51
0.5% EDTA	50.0 ± 1.4	11.3 ± 1.1	10.3 ± 0.9	0.2 ± 0.01	28.2 ± 3.41

[a] Values are means of three replicates with ± standard deviation.

of cellulase was carried out using a 2-L B. Braun stirred tank fermenter (Biostat B, Melsungen, Germany). The initial pH of the culture was adjusted to 5.5, and the culture pH was not controlled throughout the fermentation. The temperature within the fermenter was controlled at 30 °C.

The culture broth harvested from the fermenter was filtered through microfiber filter paper. The filtrate that contained cellulase was partially purified by ammonium sulfate precipitation technique (Scopes, 1988). The precipitate was resuspended in 0.05 M sodium acetate buffer (pH 5.0). Fractions from the ammonium sulfate precipitation were desalted on a column (2.5 × 30 cm) of Bio-Gel P60 equilibrated with the same buffer (flow rate ca. 70 mL/h). Fractions with cellulase activities were collected and filtered through a Minitan-S membrane ultrafiltration system (Millipore, MA) having 10 000 Da molecular mass cutoff and then dried using a freeze-dryer. The freeze-dried enzyme was redissolved in the same buffer for subsequent use in the saccharification experiments. This concentrated cellulase preparation contained 10 U/mL FPase, 285 U/mL CMCase, and 60 U/mL β-glucosidase. The rate of reducing sugar production increased with increasing OPEFB fiber concentration, and the kinetics of the enzyme reaction was estimated using the Lineweaver−Burk plot (data not shown). This cellulase has an apparent Michaelis−Menten constant (K_m) of 0.5% OPEFB fiber and a maximum velocity (V_{max}) of 22.5 μg of reducing sugars mL^{-1} min^{-1}.

Method of OPEFB Pretreatment. The OPEFB fibers obtained from the mill (Chuan Choon Group of Companies, Klang, Malaysia) was an average length of 10 cm. The OPEFB fiber was reduced to an average length of 2 cm by cutting manually using scissors. To test the suitability of OPEFB fibers as a substrate for hydrolysis, the 2 cm OPEFB fiber was further treated by various chemical and physical methods. Preliminary, the OPEFB fiber was first treated with various types of chemicals, in which 50 g of OPEFB fiber was soaked in 500 mL of different chemical solutions (0.5% v/v) [NaOH, HCl, HNO_3, ethylenediamine (EDA), ethylenediaminetetraacetic acid (EDTA)] at 30 °C for 4 h. In the subsequent experiments, the chemically treated OPEFB fibers together with the chemical solutions were autoclaved at 121 °C, 15 psi for 5 min. The effect of soaking in different concentrations of NaOH was also carried out; whereby 50 g of OPEFB fiber was soaked in 500 mL of different concentrations of NaOH (0.5 and 2%) at 30 °C for 4 h followed with autoclaving at 121 °C, 15 psi for 5 min. All the treated OPEFB fibers were filtered and washed with distilled water until no traces of acid or alkali could be detected and then dried in an oven at 95 °C for 2 days. The percentage of dry matter loss during the washing process was low (2−4%), and the different treatments imposed before washing did not significantly affect the dry weight loss. After drying, the OPEFB fibers were ground to 2 mm size using a hammer mill (Janke & Kankel, IKA-Labortechnik).

Saccharification Experiment. Enzymatic saccharification of OPEFB fibers were carried out in a shake flask incubated at 50 °C and agitated at 200 rotations/min. Saccharification was started by adding 2 mL of cellulase preparation into 18 mL of 0.05 M sodium acetate buffer, pH 5.0, containing 1 g of OPEFB fiber. Sodium azide (0.02% w/v) was added to the reaction mixture to avoid bacterial contamination. Samples were withdrawn at regular time intervals for analysis.

Analytical Procedures. Cellulose, hemicellulose, and lignin contents in untreated and treated OPEFB fibers were determined using the gravimetric method as described by Goering and Van Soest (1970).

All the activities of the three major components of cellulase (CMCase, FPase, and β-glucosidase) were determined according to the method described previously (Umikalsom et al., 1997). Reducing sugar was determined using the dinitrosalicylic (DNS) acid method (Miller, 1959) Protein content is determined by the method as described by Lowry et al. (1951) using bovine serum albumin as a standard.

The samples (0.5 mL) from the saccharification were withdrawn at different time intervals. The samples were centrifuged for 5 min at 3000*g*, and the supernatant was used for the determination of reducing sugar, glucose, and cellobiose concentration. Glucose and cellobiose concentrations were determined using ConstaMetric 3000, high-pressure liquid chromatography (HPLC) (LDC Analytical, Florida) with a refractive index detector. In the HPLC method, the mobile phase was a mixture of acetonitrile and water in a ratio of 70:30, and the flow rate was 1.0 mL/min at room temperature. The stationary phase was a prepacked 250 mm × 4 mm LiChrosorb NH-2 column. The hydrolysis (%) was calculated qualitatively according to the method described by Latiff et al. (1994) using the equation:

$$\text{hydrolysis (\%)} = [\text{reducing sugars (mg/mL)} \times 0.9 \times 100]/[\text{OPEFB (mg/mL)} \times 0.77]$$

Statistical Analysis. The results were compared by one-way analysis variance (one-way ANOVA) and treated by Duncan's multiple range test to find the differences between pretreatment means at 5% (0.05) significance level. Experimental data were analyzed using the General Linear Model Procedure of the SAS Institute Inc. (1988).

RESULTS AND DISCUSSION

Effect of the Various Treatments on Chemical Composition of OPEFB. The chemical composition of untreated and treated 2 mm OPEFB fibers with various methods is given in Table 1. The content of cellulose, hemicellulose, and lignin in untreated OPEFB

fibers were almost the same as other cellulosic materials such as wood (Parajo et al., 1995) and wheat straw (Bjerre et al., 1996).

Treatment of OPEFB fiber by soaking with various chemicals without further autoclaving did not significantly ($P > 0.05$) increase the cellulose or reduce the lignin and hemicellulose contents as compared to untreated OPEFB fiber. Autoclaving the treated OPEFB fiber with HCl, HNO_3, and NaOH at 121 °C, 15 psi for 5 min significantly ($P < 0.05$) increased the cellulose or reduced the lignin and hemicellulose contents. In general, heat treatment at a temperature above 100 °C altered the physical nature of lignin (Young et al., 1985). As lignin easily dissolves in alkaline solutions (Johansson and Ljunggren, 1994), thereby expanding its surface, the molecule becomes very accessible for oxidation. On the other hand, hemicellulose is soluble in alkali due to hydrolysis and is expected to be less stable during alkaline treatment than cellulose due to the branched hemicellulose structure as compared to the linear structure of cellulose (Saddler et al., 1993).

Although treatment of OPEFB fiber with EDA and EDTA followed by autoclaving reduced the hemicellulose content about half as compared to untreated OPEFB fiber, the cellulose and lignin contents were not significantly different ($P > 0.05$). Detroy et al. (1980) also reported that the treatment of wheat straw with 5% EDTA did not increase the cellulose content. However, the use of concentrated EDA (28%) increased the cellulose content by about 42% and affected the lignin fraction with 60% reduction. Application of cadoxen, a mixture of 28% EDA and 5% cadmium oxide, also extracts cellulose from various cellulosic materials (Ladisch, 1979). Probably, the very low concentrations of EDA and EDTA used in this study were not sufficient in altering the structure of the lignin−cellulose complex of OPEFB fiber.

Tests on the Suitability of Pretreated OPEFB Fibers as Substrate for Saccharification. *Effect of Chemical Treatment.* The time courses of the enzymatic saccharification of OPEFB fibers treated with 0.5% of different chemical solutions (NaOH, HNO_3, HCl, EDA, and EDTA) are shown in Figure 1, which also include data of untreated OPEFB fiber. In all cases, reducing sugar production was very rapid during the first 48 h, and the rate decreased toward the end of the process. The saccharification of cellulose to reducing sugar stopped after 120 h. The rate and degree of hydrolysis were varied with the different types of chemical used to treat OPEFB fiber. Among the chemicals investigated, the use of 0.5% NaOH gave the highest rate and degree of hydrolysis, followed by HNO_3, HCl, EDA, and EDTA. When OPEFB fiber treated with NaOH was used, the hydrolysis was about 2-fold higher than untreated OPEFB fiber. The degree and rate of hydrolysis using OPEFB fiber treated with EDTA was comparable to untreated OPEFB fiber.

Because the cellulose, hemicellulose, and lignin contents of OPEFB fiber treated with the different chemicals (NaOH, HNO_3, and HCl) were not significantly different ($P < 0.05$), the difference in the rate of hydrolysis may be due to changes in the structure of cellulose that led to an increase in its susceptibility to enzyme attack (Cowling, 1975). The changes in cellulose structure involved the pore structure, particle size, lignin and hemicellulose association, crystallinity, and degree of polymerization (Fan et al., 1980). The

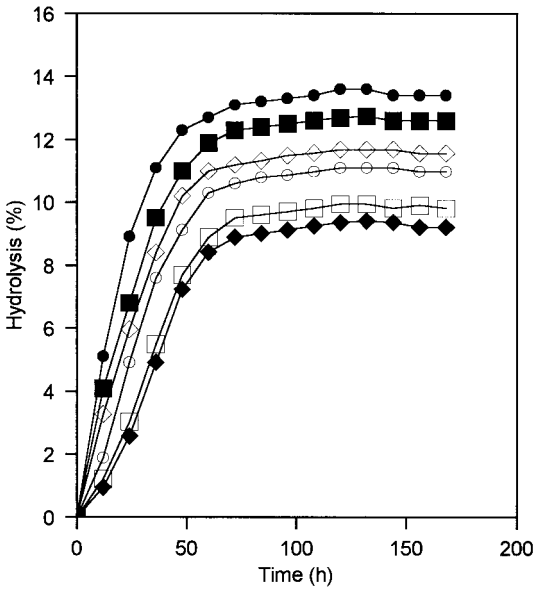

Figure 1. Time courses of saccharification of oil palm empty fruit bunch (OPEFB) fibers treated with different types of chemical. Saccharification was performed at 50 °C, pH 5.0. The percentage of hydrolysis was significantly higher ($P < 0.05$) for OPEFB fibers treated with NaOH as compared to OPEFB fibers treated with HNO_3, HCl, EDA, and EDTA. (●) NaOH; (■) HNO_3; (◇) HCl; (○) EDA; (□) EDTA; (♦) control.

intracrystalline swelling of cellulosic materials can be achieved by pretreatment with chemical reagents such as NaOH, liquid ammonia, and aliphatic amines (Detroy et al., 1980). The treatment of lignocellulosic materials with caustic soda resulted in several beneficial effects to hydrolysis such as (i) efficient delignification, (ii) open structures, (iii) removal of hemicelluloses, and (iv) ruptures of the lignin−carbohydrate bonds (Fan et al., 1981). Saccharification is enhanced as a result of delignification and structural swelling (increased in the interfacial area) and reduction of the polymerization degree and crystallinity (Fox et al., 1987).

Effect of Autoclaving. Figure 2 shows the comparison of saccharification of autoclaved and non-autoclaved OPEFB fibers treated with different chemicals. A significant increase in hydrolysis of cellulose to reducing sugar was observed when autoclaved OPEFB fibers treated with different chemicals were used as a substrate as compared to non-autoclaved OPEFB fibers. However, the highest improvement in saccharification (more than 3.5 times) was obtained when OPEFB fiber treated with NaOH was used. A slight increase in hydrolysis (less than 2 times) was observed when OPEFB fibers treated with other chemicals (HNO_3, HCl, EDA, EDTA) were used. Autoclaving of chemically untreated OPEFB fiber gave no effect to enzymatic saccharification of cellulose.

In general, autoclaving the chemically treated OPEFB fibers increased the cellulose and reduced the lignin and hemicellulose contents. However, the effect of autoclaving on cellulose, hemicellulose, and lignin contents depended on the type of chemical used for treatment (Table 1). The treatment of OPEFB fiber with alkali produced the most suitable substrate for enzymatic saccharification as compared to acid treatment. The effectiveness of the alkali treatment is expected for the following reasons. Alkali treatment causes the opening of the crystalline structure of cellulose and lignin, and lignin is decomposed to CO_2, H_2O, and carboxylic acids

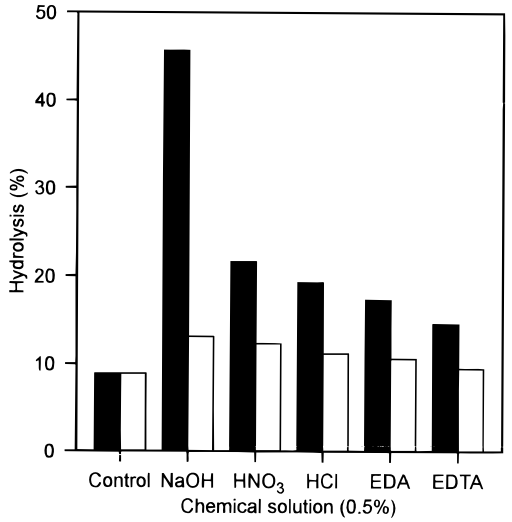

Figure 2. Effect of autoclaving chemically treated oil palm empty fruit bunch (OPEFB) fibers on the performance of the saccharification process. Saccharification was performed at 50 °C, pH 5.0. The percentage of hydrolysis was significantly higher ($P < 0.05$) for autoclaved chemically treated OPEFB fibers as compared to non-autoclaved chemically treated OPEFB fibers: (■) autoclaved; (□) non-autoclaved.

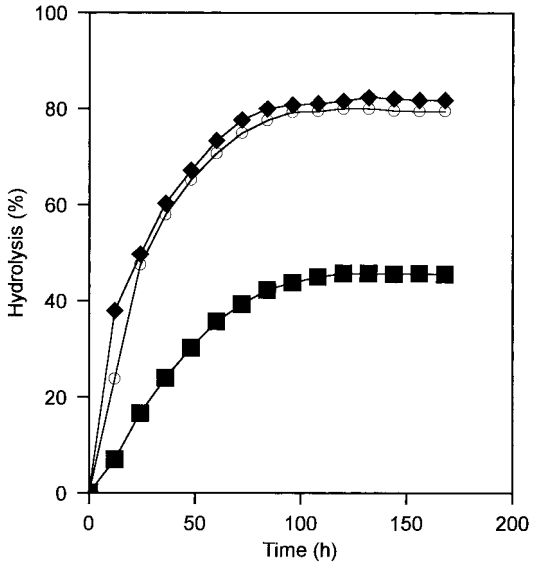

Figure 3. Effect of different concentrations of NaOH on the rate of saccharification. The percentage of hydrolysis was significantly higher ($P < 0.05$) for OPEFB fibers treated with 2% NaOH as compared to OPEFB fibers treated with 0.5% NaOH: (■) 0.5% NaOH; (○) 2% NaOH; (◆) 5% NaOH.

(Mc Ginnis et al., 1983). In addition, alkali treatment also causes the conversion of a large number of organic molecules to CO_2, H_2O, and simpler organic compounds such as low molecular weight carboxylic acids (Taylor and Weygandt, 1974). Furthermore, alkali treatment of wood materials dissolves the hemicellulose (Mc Ginnis et al., 1983).

Effect of Different NaOH Concentrations. The effect of three different concentrations of NaOH (0.5%, 2%, and 5%) in the treatment of OPEFB fiber followed by autoclaving for subsequent use in the saccharification process is shown in Figure 3. The use of concentrated NaOH (2%) increased the hydrolysis about 75% higher than the treatment with dilute NaOH (0.5%). The higher degree of delignification of OPEFB fiber with 2% NaOH as compared with 0.5% NaOH (Table 1) is the possible explanation for an increase in hydrolysis of cellulose with concentrated NaOH. However, the saccharification of OPEFB fiber treated with 2% and 5% NaOH was comparable. The use of 2% NaOH was found to be the most effective in the treatment of kallar grass straw (Latif et al., 1994). However, 0.5% NaOH was found to be more effective than 3% NaOH in the treatment of sunflower hull (Soto et al., 1994).

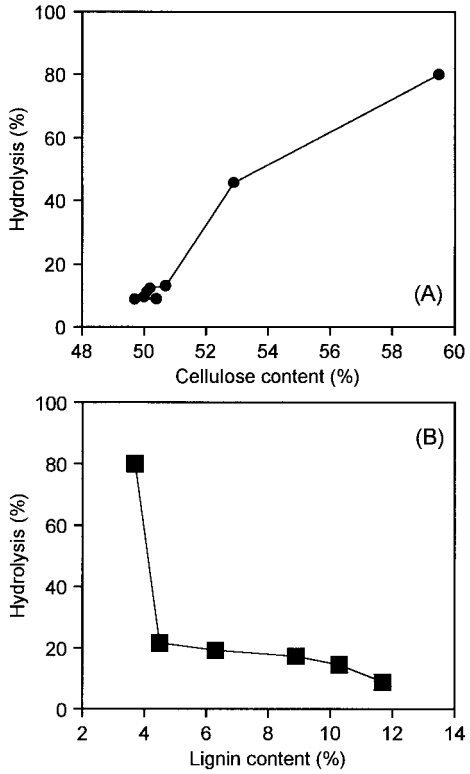

Figure 4. Relationship between (A) cellulose and (B) lignin contents on the rate and degree of saccharification.

Relationship between Cellulose and Lignin Contents and Degree of Hydrolysis. The relationship between lignin and cellulose contents and degree of hydrolysis of the chemically and physically treated OPEFB fibers is shown in Figure 4. The hydrolysis increased slightly and almost linearly with decreasing lignin content from 12% to 6.5%. However, a drastic increase in hydrolysis was observed when lignin content decreased from 6.5% to 4.5%. On the other hand, hydrolysis was increased with increasing cellulose content. However, the increase in saccharification with increasing cellulose content was not linear. This result suggests that hydrolysis did not solely depend on lignin or cellulose content but may also depend on other factors such as the pore sizes, surface area, partial decrystallinization of cellulose (Fan et al., 1980), substitution reactions with lignin−carbonium ion, and depolymerization of lignin (Ghose et al., 1983). In addition to physical alteration of lignin, autoclaving may also have other effects on the lignocellulosic materials. For example, pressure was found to be very important for the decomposition of pectin-related substances in the non-cell wall material at the alkaline conditions (Fengel and Wegener, 1989).

Comparison between Saccharification of OPEFB Fibers and Pure Cellulose. The comparison between saccharification of OPEFB fiber and commercial pure cellulose is given in Table 2. The degree of hydrolysis of pure cellulose [Avicel (microcrystalline cellulose)] was

Table 2. Yield and Productivity of Saccharification Processes Using Oil Palm Empty Fruit Bunch (OPEFB) Fibers Pretreated with Different Methods and Pure Cellulose as Substrates[a]

substrate	hydrolysis (%)	yield (g of reducing sugar/ g of substrate)	productivity (g of reducing sugar (g of substrate)$^{-1}$ h^{-1})
Avicel	12.7 ± 0.4[b]	0.12 ± 0.04	0.0017 ± 0.0004
untreated hammer-milled OPEFB	9.4 ± 0.6[a]	0.09 ± 0.006	0.0013 ± 0.0006
hammer-milled OPEFB and treated with 0.5% NaOH	13.6 ± 0.8[b]	0.13 ± 0.08	0.0019 ± 0.0008
hammer-milled OPEFB, treated with 0.5% NaOH and autoclaved at 121 °C, 15 psi for 5 min	45.7 ± 1.2[c]	0.43 ± 0.02	0.0065 ± 0.002
hammer-milled OPEFB, treated with 2.0% NaOH and autoclaved at 121 °C, 15 psi for 5 min	85.9 ± 1.5[d]	0.76 ± 0.02	0.0102 ± 0.002

[a] Values are means of three replicates with ± standard deviation. Productivity is calculated as the maximum concentration of reducing sugar produced divided by hydrolysis time. Mean values in same column with different superscripts are significantly different ($P < 0.05$).

Table 3. Comparison of Saccharification of Oil Palm Empty Fruit Bunch (OPEFB) Fibers Using Cellulase of *C. globosum* and Commercial Cellulase of *T. viride*[a]

enzyme source	maximum sugar concentration (g/L)			
	reducing sugar	glucose	cellobiose	xylose
C. globosum	38.5	16.5	3.8	18.0
T. viride	35.5	12.8	5.6	17.1

[a] Standard deviation of analysis in triplicate were in the range of 2−3% of the measured values. Saccharification was carried out at 50 °C for 72 h, pH 5.0.

comparable to untreated OPEFB fiber. On the other hand, hydrolysis of autoclaved OPEFB pretreated with 2.0% NaOH was about 7 times higher than the hydrolysis of Avicel. The higher improvement in yield of saccharification using treated OPEFB fiber was achieved in this study as compared to those reported in the literature (Kumakura, 1997; Parajo et al., 1995; Waldron and Eveleigh, 1986). In comparison with the untreated OPEFB fiber, autoclaved fiber treated with 2% NaOH improved the yield in term of reducing sugar produced based on dry OPEFB consumed by about 8 times (Table 2). The maximum productivity (0.0102 g of reducing sugars (g of OPEFB)$^{-1}$ h)$^{-1}$ obtained from hammer-milled OPEFB fiber, treated with 2.0% NaOH and autoclaved at 121 °C, 15 psi for 5 min, was in the range of those obtained from other lignocellulosic materials treated with dilute alkali (0.01−0.3 g of reducing sugars (g of substrate)$^{-1}$ h)$^{-1}$ using fungal cellulases (Parajo et al., 1995; Latiff et al., 1994; Dekker and Wallis, 1983; Detroy et al., 1980).

The greater resistance of agrowaste lignocellulosic materials to enzymatic saccharification than pure cellulose may be attributed chiefly to their lignin content (Okeke and Obi, 1994). Naturally, lignin encrust cellulose and hemicellulose, rendering them resistant to enzymatic saccharification (Kirk and Farrel, 1987). Other factors that hinder enzymatic saccharification of lignocelluloses include crystallinity, the degree of polymerization, and the specific surface area (Puri, 1984).

Comparison betwen Saccharification of OPEFB Fibers Using Cellulase of *C. globosum* and Commercial Cellulase. The profile of sugars during saccharification of OPEFB fibers using cellulase of *C. globosum* and commercial cellulase of *T. viride* (Fluka, Chemie AG, Switzerland) is given in Table 3. The commercial cellulase used in this study contained 16 U/mL FPase, 960 U/mL CMCase, and 21 U/mL β-glucosidase. The HPLC analysis of the soluble product of saccharification using the autoclaved OPEFB fibers treated with 2% NaOH revealed that the predominant reducing sugar was glucose. The reducing sugar and glucose produced during saccharification using cellulase of *C. globosum* was slightly higher than was obtained with commercial cellulase. The higher β-glucosidase activity in the cellulase of *C. globosum* would be expected to a rapid conversion of cellobiose to glucose (Ghani and Rickard, 1990). In addition, the high activity of β-glucosidase in cellulase also helps to circumvent the inhibitory effect of cellobiose accumulation (Woodward and Wiseman, 1982).

The physical and chemical pretreatments should be involved in the preparation of suitable OPEFB fibers as a substrate for enzymatic saccharification. From the result of this study, it can be suggested that hammer-milled OPEFB (2 mm fiber length) soaked in 2.0% NaOH for 4 h and then autoclaving at 121 °C, 15 psi for 5 min was found to be the best combination of pretreatments for preparation of suitable OPEFB fibers for enzymatic saccharification. The improvements in hydrolysis with these steps of pretreatment are given in Table 2. The hydrolysis increased significantly ($P < 0.05$) with the increasing step of pretreatment. The lower rate and degree of hydrolysis when untreated OPEFB fiber was used may be due to high proportions of cellulose and hemicellulose presence in the residue that could not be accessible to the cellulase enzyme. The untreated OPEFB fiber still contained lignocellulosic in the native form in which cellulose was embedded in the hemicellulose and lignin matrix.

CONCLUSIONS

The pretreatment of OPEFB fiber with alkali (NaOH) was found to be more efficient for the preparation of a suitable substrate for the saccharification process as compared to acids (HCl and HNO_3). Autoclaving the chemically treated OPEFB fibers at 121° C, 15 psi for 5 min improved hydrolysis about 2-fold. Improvement in hydrolysis with chemical pretreatment and autoclaving was associated with the reduction in hemicellulose and lignin content and the increase in cellulose content. The cellulase of *C. globosum* was able to hydrolyze the autoclaved OPEFB fiber pretreated with 2% NaOH up to about 85.9% hydrolysis, which gave an overall productivity of 0.0102 g of reducing sugar (g of OPEFB)$^{-1}$ h^{-1}, respectively.

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