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Effect of physical, chemical and thermal pretreatments on the enzymatic hydrolysis of oil palm empty fruit bunch (OPEFB)

(Kesan prarawatan fizikal, kimia dan termal terhadap hidrolisis enzimitik tandan kosong kelapa sawit)

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Key words: oil palm empty fruit bunch (OPEFB), pretreatment, hydrolysis, reducing sugars

Abstract

The effect of physical, chemical and thermal pretreatments on the chemical composition (cellulose, hemicellulose and lignin) and the physical structure of oil palm empty fruit bunch (OPEFB) fibres for subsequent use in reducing sugar production were investigated. Physical pretreatment was done to reduce the size of OPEFB by grinding the OPEFB to 1–2 cm followed by hammer-milling to an average size of 1–2 mm. Chemical pretreatment was done by soaking the OPEFB fibres in 0.1 M and 0.5 M NaOH. Thermal pretreatment was done by two methods: (i) heating at 121 ºC, 15 psi for 15 min and (ii) heating at 240 ºC, 40 psi for 1 h and 50 min. The effect of combined pretreatments (chemical, physical and thermal) was also studied. From the results, it was evident that the cellulose, hemicellulose and lignin composition in the OPEFB were affected. Higher cellulose content was found in treated OPEFB as compared to untreated OPEFB due to the removal of lignin. The scanning electron microscope (SEM) micrograph showed a marked change in the physical appearance of treated OPEFB as compared to the untreated OPEFB, especially OPEFB treated with combined pretreatments. Enzymatic saccharification of OPEFB treated with various pretreatments was conducted for reducing sugar production using Celluclast 1.5 litres (Novozyme A/S, Denmark). Saccharification was conducted in shaker incubator at 37 ºC, 180 rpm. Hammer-milled OPEFB fibres soaked in 0.5 M NaOH and heated at 121 °C, 15 psi gave the highest conversion yield in this study with 0.53 g sugars/g OPEFB being produced. This was nine-fold higher as compared to chemically and thermally untreated OPEFB fibres (1–2 cm) where only 0.06 g sugars/g OPEFB was produced.

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Introduction

Bioconversion is defined as the use of biological processes to transform biomass materials from one form to another. These involve the use of enzymes, microbes or other biological agents, alone or in combination. Converting lignocellulosic materials to valuable products is an example of bioconversion. Examples of valuable products from lignocellulosic materials are sugars, acetone-butanol-ethanol (ABE), organic acids and polymers.

In Malaysia, oil palm plantation is the biggest agriculture business. Malaysia is one of the largest palm oil producers in the world. The production of palm oil continues to increase annually. High production of crude palm oil generates abundant lignocellulose solid wastes, namely oil palm empty fruit bunch (OPEFB), oil palm fruit fibre and oil palm shell. In 2005, it was estimated that 17.6 million tonnes of OPEFB was generated at the mills. This implies an abundant supply of OPEFB, a readily available carbon source for bioconversion.

Umi Kalsom (1997) studied the potential of OPEFB as substrate for cellulase production. More than 50% of the OPEFB component is cellulose; therefore it has high potential to be bioconverted. However, the problem in bioconversion of lignocellulosic materials is the difficulty of getting high yield of reducing sugars as the end product due to complex structure and component.

Lignocellulosic material comprises three major components with cellulose as the largest component (40–50%), followed by hemicellulose which contributes 20–30% of the overall components and finally lignin with only 15–20% of biomass (Lasure and Zhang 2004). Of the three components, lignin is the most recalcitrant to degradation whereas cellulose, because of its highly crystalline structure, is more resistant to hydrolysis than hemicellulose (Howard et al. 2003). Lignin is further linked to both hemicellulose and cellulose forming a physical seal around the latter two components that is an impenetrable barrier preventing penetration of solutions and enzymes. Hence, it is very difficult for the enzyme to access the cellulose and hemicellulose.

The three major factors that determine the effectiveness of chemical, enzymatic or biological conversion process are lignin and hemicellulose content, cellulose crystallinity and pore size or accessible surface area. Therefore, pretreatment is needed in order to enhance sugar production from lignocellulosic materials.

The objective of this study was to determine the effects of physical, chemical and thermal pretreatments on the chemical composition and structure of OPEFB, and the subsequent reducing sugar production. Combinations of these pretreatments were also studied. In this study, only NaOH was used in chemical treatment as based on the previous finding by Umi Kalsom et al. (1997), NaOH gave the highest impact on cellulose, hemicellulose and lignin content in OPEFB as compared to other chemicals such as HNO_3, HCl, EDA and EDTA.

Materials and methods

The raw material used in this study was shredded OPEFB obtained from Sri Ulu Langat Palm Oil Mill at Dengkil, Selangor. The OPEFB was soaked in detergent to remove any residual oil and then dried in an oven at 105 ºC overnight.

Physical pretreatment

OPEFB was ground by a grinder. The ground OPEFB fibres would have an average size of 1–2 cm. To compare the effect of size reduction, the ground OPEFB fibres were further treated by hammer mill (Janke & Kunkel) to an average size of 1–2 mm. Ground OPEFB (1–2 cm) was only used to compare the effect of size. For subsequent treatments only fine, hammer-milled OPEFB fibres (1–2 mm) were used.

Chemical pretreatment

Chemical pretreatment was done by soaking the hammer-milled OPEFB fibres in NaOH

for 4 h. Two molarities of NaOH were used i.e. 0.1 M and 0.5 M. Following that, the fibres were soaked in tap water overnight and washed until all the alkali used was removed. The fibres were then dried in an oven at 105 ºC overnight.

Thermal pretreatment

The effect of thermal pretreatment on the production of reducing sugars was studied by comparing OPEFB fibres**,** which have been thermally treated**,** by using two different steam-sterilisation set-ups, i.e. low temperature and high temperature. In the first set-up, hammer-milled OPEFB fibres were treated by soaking the fibres in distilled water and then autoclaved at 121 ºC, 15 psi for 15 min. The second set-up involved heating the shredded OPEFB in the steriliser at the mill, at 240 ºC, 40 psi for 1 h and 50 min. The OPEFB were then dried in an oven at 105 ºC overnight. Steam-sterilised OPEFB (high temperature) were then hammer-milled to the size similar to that of the steam-sterilised OPEFB (low temperature).

Combined pretreatments

Combination of pretreatments was done by combining the three treatments, i.e. physical, chemical and thermal pretreatment on the OPEFB fibres. Hammer-milled OPEFB was soaked in 0.1 M and 0.5 M NaOH for 4 h. The OPEFB was then heated at 121 ºC, 15 psi for 15 min. Following that, the treated OPEFB fibres were soaked in tap water and washed until all the residual alkali was removed. The fibres were then dried in an oven at 105 ºC overnight.

Enzymatic saccharification

Saccharification was done by using 1% (w/v) of treated and untreated OPEFB fibres in distilled water with 0.5 ml of cellulase (Celluclast 1.5 litres) from Novozymes A/S, Denmark). The cellulase contained 56.5 U/ml FPase, 147.5 U/ml CMCase and 16.8 U/ml β-glucosidase. 1 unit of FPase and CMCase activity was expressed as 1 μmole of glucose liberated per ml enzyme per minute, while 1 unit of β-glucosidase activity was determined as 1 μmole of p-nitrophenol liberated per ml enzyme per minute.

The saccharification was done in a shaker incubator at 200 rpm at 37 ºC. Samples were withdrawn at intervals for 48 h. Samples were then centrifuged at 2,800 g for 15 min. The supernatant was analysed for reducing sugar content. Percentage of hydrolysis was calculated based on the amount of reducing sugars obtained over cellulose content in the OPEFB.

Analytical procedures

The determination of cellulose, hemicellulose and lignin content in untreated and treated OPEFB fibres were performed following the standard methods of AOAC Official Method 973.18 (AOAC 1997). Reducing sugars were determined using method proposed by Wang (2003). In this method, 2 ml of diluted sample was added to 3 ml of DNS and boiled for 15 min. After boiling, 1 ml of Rochelle salt was added. The absorbance was recorded at 575 nm using spectrophotometer against the blank of distilled water.

SEM micrograph

SEM analysis was conducted using SEM model JEOL 6400. For sample preparation, the fibres were dried and mounted onto the stub. Gold coating was then taken place in sputter coater.

Results and discussion

Effect of various pretreatments on the chemical composition of OPEFB

A trend was observed where the composition of cellulose was increased in the treated OPEFB, while lignin and hemicellulose composition was decreased (*Table 1*). The cellulose, hemicellulose and lignin content in untreated OPEFB fibre were similar to that reported by Rushdan (2002), who studied the chemical composition of alkaline

Table 1. Cellulose, hemicellulose and lignin composition of treated and untreated OPEFB

Pretreatment	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Untreated OPEFB (only physically treated)			
1) Ground OPEFB (1–2 cm)	51.22	28.24	15.19
2) Hammer-milled OPEFB (1–2 mm)	51.28	28.18	15.17
Chemical treatment			
3) Hammer-milled OPEFB + 0.1 M NaOH	51.37	27.57	14.21
4) Hammer-milled OPEFB + 0.5 M NaOH	53.95	25.49	13.20
Thermal treatment			
5) Low temperature, T (121 °C)	51.49	27.00	14.08
6) High temperature, T (240 °C)	54.67	25.70	13.01
Combination of pretreatments			
7) 0.1 M NaOH + thermal (low T)	54.42	23.36	13.70
8) 0.5 M NaOH + thermal (low T)	66.61	16.80	10.47
9) Thermal (high T) + 0.1 M NaOH + thermal (low T)	59.39	22.34	13.42
10) Thermal (high T) + 0.5 M NaOH + thermal (low T)	67.30	18.55	10.88
11) Thermal (low T) + 0.1 M NaOH	52.56	26.96	14.33
12) Thermal (low T) + 0.5 M NaOH	58.55	23.97	13.18
13) Thermal (high T) + 0.1 M NaOH	52.80	27.59	13.97
14) Thermal (high T) + 0.5 M NaOH	57.90	22.14	13.56

pulps from OPEFB**,** and Mohamad et al. (1999) who studied the potential of OPEFB in sugar production.

In the chemically treated OPEFB, different molarities of NaOH (0.1 M and 0.5 M) showed significant effect on the chemical composition. OPEFB treated with 0.5 M NaOH exhibited higher cellulose content, but lower in hemicellulose and lignin content as compared to OPEFB treated with 0.1 M NaOH. The differences of the cellulose, hemicellulose and lignin composition in the two treatments were 2.6, 2.1 and 1.0% (w/w), respectively.

For thermal treatment, cellulose content was higher in steam-sterilised OPEFB using high temperature. Both hemicellulose and lignin content in OPEFB were decreased when thermal pretreatment was conducted. This shows that high temperature not only promoted delignification (removal of lignin), but also removal of hemicellulose. The decrement in hemicellulose and lignin content was bigger when higher temperature was used. The differences of the cellulose, hemicellulose and lignin content in steam-sterilised OPEFB (low and high temperatures) were 3.2, 1.3 and 1%, respectively.

Combination of pretreatments gave a prominent increment in cellulose content and reduction of lignin and hemicellulose content. The highest cellulose content was in OPEFB treated with 0.5 M NaOH followed by thermal treatment. This shows that combination of chemical and thermal treatment gave superior result. Based on the results, OPEFB treated twice in thermal treatment, i.e. heated in steriliser (240 ºC) followed by heating in autoclave at 121 ºC, did not give significant effect towards its cellulose and lignin content. Cellulose content in double-thermal treatment OPEFB was 67.3% (w/w) as compared to 66.6% (w/w) for single-thermally treated OPEFB. As synergistic effect of the chemical and thermal pretreatments was proven to cause better alteration in the OPEFB chemical composition, hence higher temperature is not needed when chemical pretreatment is used in combination.

Effect of various pretreatments on OPEFB structure

The effect of pretreatments on OPEFB structure was studied using SEM micrograph. Marked difference can be seen on the structure of untreated (*Plates 1a–b*) and treated OPEFB (*Plates 1c–h*), especially OPEFB treated with combination of pretreatments (*Plates 1g–h*). In the untreated OPEFB (*Plates 1a–b*), the observation shows the OPEFB structure as a big, single strand of fibre. Comparison of two different sizes of OPEFB, i.e. 1–2 mm and 1–2 cm as shown in *Plates 1a–1b* revealed that there was no difference in the structure of the two OPEFB. Both figures show the same OPEFB morphology. Therefore based on the SEM study, it can be concluded that size reduction did not bring marked effect on the structure of OPEFB.

To compare the effect of pretreatments on the OPEFB structure, *Plate 1a* was used as reference since in all pretreatments; fine, hammer-milled OPEFB fibres (1–2 mm) were used. Thermally treated OPEFB in *Plates 1c–d* show an altered structure where the initial big, single strand and tightly packed of untreated OPEFB was replaced with a loosened structure of OPEFB (OPEFB fibre strands can be seen in thermally treated OPEFB, especially in *Plate 1d* where higher temperature was used).

In lignocellulose, lignin acts like a glue by filling the spaces between and around cellulose and hemicellulose (Tina 1998). Therefore, the loosening OPEFB structure suggested the occurrence of delignification (lignin removal). In comparing the effect of different temperature used in thermal pretreatment, it was seen that OPEFB heated at 240 ºC, had more lignin removal as compared to low temperature (121 ºC). This shows that higher temperature used promotes delignification.

Plates 1e–f illustrate the effect of chemical pretreatment on the OPEFB structure. As compared to the untreated OPEFB in *Plate 1a*, both *Plates 1e* and *1f* show alteration in their structure. Delignification was seen to occur in both plates, similar to that observed in *Plate 1c*. Therefore, it can be concluded that chemical pretreatment by NaOH may cause delignification. Besides delignification, chemically treated OPEFB fibres in *Plates 1e* and *1f* show porous structure. The presence of pores is believed to be due to the removal of hemicellulose. This is in agreement with Palonen (2004) who reported that, chemically-pretreated lignocellulose has more fractions of pores that are accessible for enzymatic attack, and the increment was due to the removal of hemicellulose. Hence, it was evident that hemicellulose was removed during chemical pretreatment.

Combination of pretreatments gave the biggest impact in alteration of OPEFB structure. *Plates 1g* and *1h* show the effect of combining pretreatments on the OPEFB structure. The structure observed in both figures was totally different as in untreated OPEFB as shown in *Plate 1a*. In *Plates 1g* and *1h*, a distinctive observation on the effect of hemicellulose removal and delignification can be seen. The appearance of hollows and the loosening structure of OPEFB fibres which could be seen by strand formation are clearly showed in both plates. *Plate 1h* gives a better view as in that figure, the OPEFB was treated by 0.5 M NaOH compared to *Plate 1g* treated by 0.1 M NaOH.

As mentioned previously, strand formation is an indication that the structure has been loosened up due to delignification. Since lignin acts as glue and often referred to as the plant cell wall adhesive, thus when delignification process occurred, the bonding between cellulose strands loosened. Hemicellulose removal was also occurred as the pores are clearly seen in both plates.

In short, pretreatments were found to alter the structure of the OPEFB and may affect the subsequent reducing sugar production. Single pretreatment was found to affect the structure of the OPEFB but was not as superior as the combination

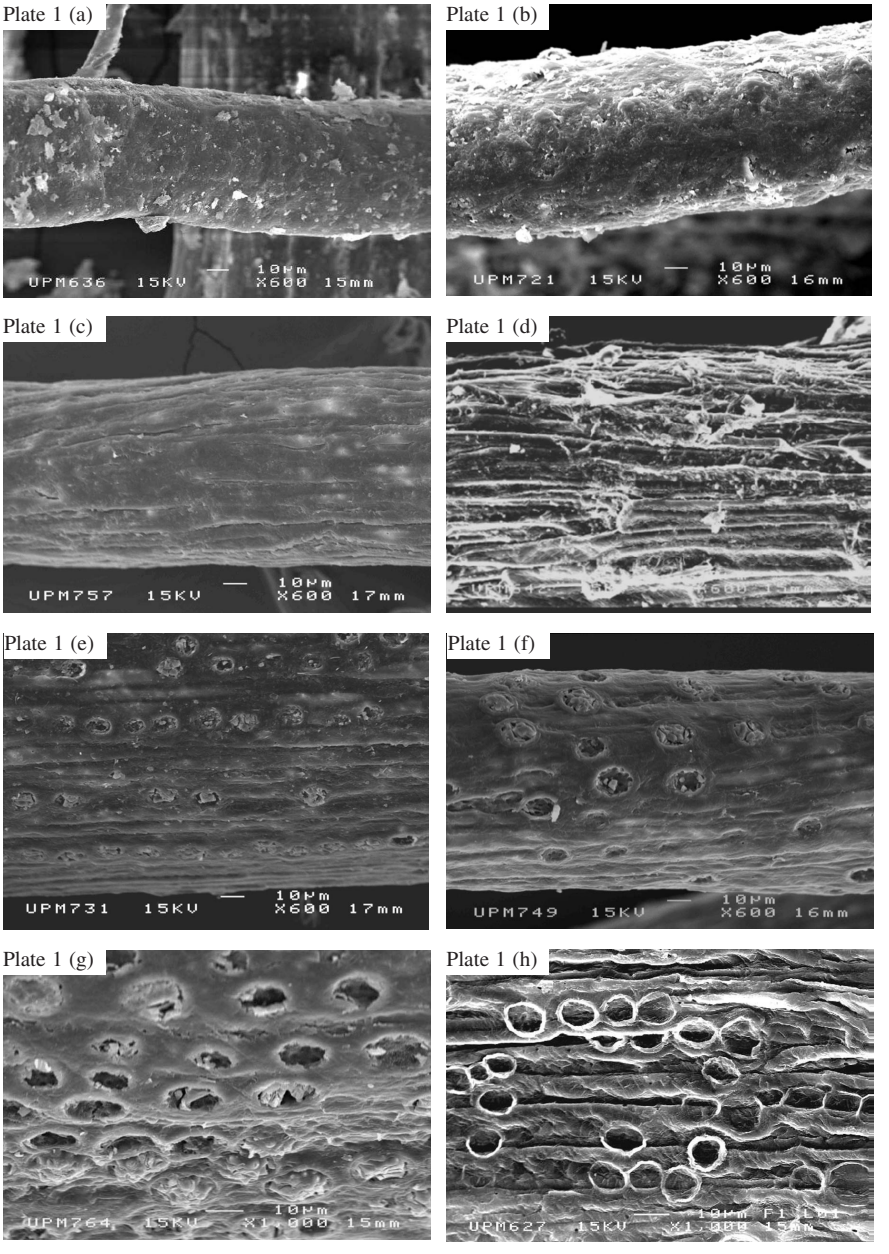

Plate 1. SEM image of OPEFB fibres (magnification x600 and x1000). (a) Hammer-milled OPEFB (1–2 mm), (b) Ground OPEFB (1–2 cm), (c) steam-sterilised OPEFB (low T), (d) steam-sterilised OPEFB (high T), (e) OPEFB treated with 0.1 M NaOH, (f) OPEFB treated with 0.5 M NaOH, (g) OPEFB treated with 0.1 M NaOH + heated (low T), (h) OPEFB treated with 0.5 M NaOH + heated (low T)

of pretreatments step. Combination of pretreatments gave the biggest impact toward its structure, particularly for those treated with 0.5 M NaOH followed by heating in autoclave at 121 ºC, 15 psi at 15 min.

Effect of various pretreatments on reducing sugar production

Higher reducing sugar production was obtained when combination of pretreatments was applied to the OPEFB (*Table 2*). The effect of physical treatment by mean of size reduction of OPEFB fibres from the average of 1–2 cm to the average of 1–2 mm did not show any marked difference whereby only an increment of 0.15 g/litre of total reducing sugars produced. Even though the particle size was reduced, restriction towards enzymatic attack still occurs due to the presence of lignin in the structure as judged in *Plate 1b*. This resulted in low reducing sugar production.

Reducing sugars produced by untreated OPEFB (1–2 mm) after 48 h of saccharification was 0.73 g/litre, with the yield of 0.07 g reducing sugars/g OPEFB. This result is competitive with the result obtained by Kader et al. (1999), where at the 48th hour, total reducing sugars produced by OPEFB was 0.65 g/litre. The same enzyme was used, i.e. Celluclast 1.5 litres from Novozymes A/S, Denmark.

For chemical pretreatment, OPEFB fibres treated with 0.5 M NaOH produced higher reducing sugars as compared to OPEFB treated with 0.1 M NaOH and the difference was about two-fold. The yield for reducing sugars produced from OPEFB treated with 0.5 M NaOH was 0.16 g/g substrate, while the yield of reducing sugars produced from OPEFB treated with 0.1 M NaOH was 0.09 g/g substrate. This was expected as more alkaline molarity was used in the 0.5 M NaOH than in the 0.1 M. These results are in agreement with the previous

Table 2. Yield of saccharification processes using treated and untreated OPEFB

Pretreatments	Hydrolysis (%)	Reducing sugars (g/litres)	Yield (g of reducing sugars/g of substrate)
Physical treatment			
1) Ground OPEFB (1–2 cm)*	8.65 ± 0.7	0.58 ± 0.17	0.06 ± 0.017
2) Hammer-milled OPEFB (1–2 mm)	9.19 ± 0.2	0.73 ± 0.02	0.07 ± 0.002
Chemical treatment			
3) Hammer-milled OPEFB + 0.1 M NaOH	11.15 ± 1.2	0.88 ± 0.12	0.09 ± 0.012
4) Hammer-milled OPEFB + 0.5 M NaOH	19.51 ± 0.3	1.55 ± 0.03	0.16 ± 0.003
Thermal treatment			
5) Low temperature, T (121 ºC)	16.31 ± 0.2	1.28 ± 0.02	0.13 ± 0.002
6) High temperature, T (240 °C)	14.43 ± 0.5	1.16 ± 0.05	0.12 ± 0.005
Combination of pretreatments			
7) 0.1 M NaOH + thermal (low T)	44.61 ± 1.3	3.47 ± 0.13	0.35 ± 0.013
8) 0.5 M NaOH + thermal (low T)	63.54 ± 2.6	5.30 ± 0.26	0.53 ± 0.026
9) Thermal (high T) + 0.1 M NaOH + thermal (low T)	26.92 ± 0.3	2.20 ± 0.03	0.22 ± 0.003
10) Thermal (high T) + 0.5 M NaOH + thermal (low T)	64.57 ± 2.6	5.54 ± 0.26	0.55 ± 0.026
11) Thermal (low T) + 0.1 M NaOH	9.68 ± 0.2	0.77 ± 0.02	0.08 ± 0.002
12) Thermal (low T) + 0.5 M NaOH	14.18 ± 0.8	1.17 ± 0.08	0.12 ± 0.008
13) Thermal (high T) + 0.1 M NaOH	15.17 ± 1.1	1.22 ± 0.11	0.12 ± 0.011
14) Thermal (high T) + 0.5 M NaOH	20.24 ± 2.0	1.62 ± 0.20	0.16 ± 0.020

*Ground OPEFB was used only to compare the effect physical treatment. For other experiments, hammer-milled OPEFB (1–2 mm) was used

study where an increase in hydrolysis of cellulose was observed when high concentrated NaOH was used (Umi Kalsom et al. 1997).

For thermal pretreatment, although SEM micrographs show an obvious effect of the two pretreatments as in *Plates 1c–d*, the results in *Table 2* showed that there was no marked difference in reducing sugar production. In fact, the amount of hydrolysed holocellulose in steam-sterilised OPEFB (high temperature) was lower by 1.9% (w/w) than those undergo steam-sterilisation (low temperature). Calculation was based on the total cellulose and hemicellulose content (holocellulose). About 16.3% of total holocellulose was hydrolysed in the sample treated at high temperature, while at low temperature only 14.4% of total holocellulose was hydrolysed. This could be due to the increment of crystalline cellulose in the double-thermal treated OPEFB as according to Umi Kalsom (1997), thermal pretreatment tends to cause the amorphous region of cellulose to crystallise.

The effect of synergism by combining the physical, chemical and thermal pretreatments on reducing sugar production was investigated. Overall, combination of pretreatments resulted in an improvement in the reducing sugar production. Combination of pretreatments has more advantages because the effects of each pretreatment are combined. Delignification, hydrolysis of hemicellulose, decreased cellulose crystallinity index and alteration of lignocellulosic material, all occurred at once. Delignification allows enzymatic and microbiological access to the cellulose, while reduction in cellulose crystallinity increases the rate of all three modes of attack of cellulases on the cellulose. Enhanced cellulose accessibility can be achieved by hemicellulose removal because the relative ease of hemicellulose hydrolysis provides an ideal avenue for creating larger pores in the microfibrils (Tina 1998). Removal of hemicellulose results in an increase in both the accessible pore volume and the specific surface area (Palonen 2004).

The highest reducing sugar production was observed from the OPEFB sample treated in pretreatment no. 10 where the reducing sugars produced was 0.55 g/g OPEFB with the hydrolysis percentage of 64.57% (w/w) (*Table 2*). This was followed by pretreatment no. 8. The reducing sugars produced were 0.53 g/g OPEFB and the hydrolysis percentage was 63.54%. The difference of reducing sugars produced between the two treatments was only 0.2 g/g. Pretreatment no. 10 involved double-thermal pretreatments while pretreatment no. 8 only involved single-thermal pretreatment. Therefore, pretreatment no. 8 is preferred due to its effectiveness in increasing hydrolysis of cellulose and is economically efficient. Although pretreatment no. 10 enhanced reducing sugar production, it was not economically efficient due to high energy needed to generate the two sterilisation set-ups.

Overall, hydrolysis of OPEFB to sugars by using Celluclast 1.5 litres was not very high. The percentage of hydrolysis of the OPEFB obtained was about 64% with 0.53 g reducing sugars/g OPEFB. Umi Kalsom (1997) reported higher conversion of reducing sugars from OPEFB when cellulase from *Chaetomium globosum kunze* was used (0.76 g reducing sugars/g OPEFB).

In this study, commercial enzyme Celluclast 1.5 litres was used, which is a commercially available liquid cellulase prepared from the fungus *Tricoderma reesei* (Ana et al. 2004). Kader et al. (1999) found that *T. reesei* has a low β-glucosidase activity. Therefore, the low hydrolysis is may be due to low β-glucosidase activity in the enzyme.

Enzymatic assay on Celluclast 1.5 litres found that the enzyme has a β-glucosidase activity of 16.8 U/ml. Low β-glucosidase activity accumulates cellobiose, an intermediate of cellulose hydrolysis

which is an inhibitor of the cellulases. To overcome the problem, it is proposed that a combination of cellulases from *T. reesei* and *Aspergillus niger* is used instead. Cellulase from *A. niger* has high β-glucosidase activity. This is in agreement with Hassan et al. (2001) where Novozyme 188, cellulase from *A. niger* has β-glucosidase activity of 168 U/m*l* which was ten-fold as compared to that in Celluclast 1.5 litres.

Effect of sequence of pretreatment in combined pretreatments of OPEFB fibres

As being discussed previously, combination of chemical and thermal pretreatments gave superior effect on reducing sugar production from OPEFB. Sequence of pretreatment in combined pretreatments method may cause different effect. Anne et al. (2000) found that the additional of base during thermal treatment improved the chemical composition of lignocellulose.

In the same combination of chemical and thermal pretreatments, the sequence of pretreatment caused a marked difference in the two techniques. The first technique involved chemical pretreatment by soaking the OPEFB in NaOH (0.1 M and 0.5 M) for 4 h followed by thermal pretreatment by heating the OPEFB at 121 °C, 15 psi for 15 min. The second technique was to start with thermal pretreatment and then followed by soaking in NaOH.

The first technique (pretreatment no. 7 and 8) produced more reducing sugars as compared to the second technique (pretreatment no. 11 and 12) (*Table 2*). The increment of hydrolysis percentage was 4.5 fold. In the first technique, the OPEFB fibres were soaked in NaOH for 4 h at ambient temperature. Then, the fibres were heated chemically, with the NaOH. In the second experiment, the fibres were first heated, followed by soaking in NaOH at ambient temperature. From these results, it showed that effective thermal treatment required the presence of NaOH. This explains higher reducing sugars being produced in the first technique.

Conclusion

Combination of pretreatments that is by soaking the OPEFB fibres in 0.5 M NaOH and autoclaving at 121 °C and 15 psi is the best way in altering the physical structure and chemical composition of the OPEFB, as well as in reducing sugar production. Reducing sugars produced from this method was 5.30 g/litres with 64% hydrolysis. Apart from combination of pretreatments, the sequence of pretreatment in combined pretreatment methods gave a high impact on reducing sugar production. OPEFB treated by chemical treatment followed by thermal treatment was best produced the reducing sugars as compared to the reversed-pretreatment technique.

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References

Ana, M.L., Krisztina, G., Aernout, A.M., Bernadet, A.M.R., Pieternel, A.M., Claassen., John, V.D.O. and Willem, M.D.V. (2004). Substrate-induced production and secretion of cellulases by *Clostridium acetobutylicum*. *Applied and Environmental Microbiology 70(9):* 5238–5243

Anne, B.T., Vibeke, B., Kristina, V.N., Bodil, P. and Michael, S.J. (2000). Effects of chemical-physical pretreatment processes on hemp fibres. *Bioresource Hemp:* 1–6

AOAC (1997). Official Method 973.18, Fiber (Acid Detergent) and Lignin in Animal Feed. *In: Official Methods of Analysis of AOAC International.* 16th ed., p. 28–29. Arlington, VA: AOAC International

Hassan, M.A., Rahman, N.A., Karim, M.I.A., Shaari, A. and Ariff, A. (2001). Bioconversion of solids from palm oil mill effluent to sugars for the production of polyhydroxyalkanoates. *NEDO Seminar,* Kyoto

Howard, R.L., Abotsi, E., Jansen van Rensburg, E.L. and Howard, S. (2003). Lignocellulose biotechnology: issues of bioconversion and enzyme production. *African J. Biotech. 2(12):* 602–619

Kader, J., Getha, K., Wan, M. and Othman, O. (1999). Hydrolysis of empty fruit bunch of oil palm using cellulolytic enzymes from

Aspergillus terreus IMI 28243. *J. Microbiol. Biotechnol. 9(4):* 514–517

Lasure, L.L. and Zhang, M. (2004). Bioconveraion and biorefineries of the future. Retrieved on 25 Sept. 2004 from http://www.pnl.gov/biobased/docs/biorefineries.pdf

Mohamad, S., Mohamadiah, B., Rosnah, M.S., Wan Zailan, W.O., Mahzan, M., Wan Hasamudin,W.H. and Abd Gapor, M.T. (1999). The potential of lignocellulosic materials from oil palm industry for sugar production. *Oil Palm Bulletin:* 262–266

Palonen, H. (2004). Role of lignin in the enzymatic hydrolysis of lignocellulose. Dissertation for the degree of Doctor of Technology, Helsinki University of Technology, Finland

Rushdan, I. (2002). Chemical composition of alkaline pulps from oil palm empty fruit bunches. *Oil Palm Bulletin 44:* 19–24

Tina, J. (1998). Steam explosion pretreatment of cotton gin waste for fuel ethanol Production. Master Thesis Virginia Polytechnic Institute and State University, United States of America

Umi Kalsom, M.S. (1997). Optimization of cellulase production by *Chaetomium globosum* strain 414 using oil palm empty fruit bunch fibre as substrate, PhD Thesis, Universiti Putra Malaysia, Malaysia

Umi Kalsom, M.S., Ariff, A.B. and Zulkifli, H.S. (1997). The treatment of oil palm empty fruit bunch fibre for subsequent use as substrate for cellulase production by *Chaetomium globosum Kunze*. *J. Bioresource Tech. 62:* 1–9

Wang, N.S. (2003). Glucose assay by Dinitrosalicylic colorimetric method. Retrieved on 25 Sept. 2004 from http://www.glue.umd.edu/~NSW/ench485/lab4a.htm

Abstrak

Kesan prarawatan fizikal, kimia dan terma terhadap komposisi kimia (selulosa, hemiselulosa dan lignin) serta struktur fizikal gentian tandan kosong kelapa sawit (OPEFB) untuk digunakan dalam penghasilan gula penurun telah dikaji. Prarawatan fizikal telah dilakukan untuk mengecilkan saiz OPEFB dengan cara mengisar OPEFB kepada saiz 1–2 cm dan diikuti dengan proses pengisaran untuk mendapatkan saiz purata 1–2 mm. Prarawatan kimia telah dilakukan dengan cara merendam gentian OPEFB di dalam 0.1 M dan 0.5 M NaOH. Prarawatan termal dilakukan dengan menggunakan dua kaedah: (i) pemanasan pada suhu 121 °C , 15 psi selama 15 minit dan (ii) pemanasan pada suhu 240 °C, 40 psi selama 150 minit. Kesan gabungan prarawatan (fizikal, kimia dan termal) juga dikaji. Hasil keputusan kajian ini membuktikan bahawa komposisi selulosa, hemiselulosa dan lignin telah menunjukkan kesan. Kandungan selulosa yang lebih tinggi didapati dalam OPEFB yang dirawat berbanding dengan tidak dirawat disebabkan oleh penguraian lignin. Mikrograf yang dihasilkan oleh mikroskop penganalisis elektron (SEM) menunjukkan perubahan aras fizikal yang ketara di dalam OPEFB yang dirawat berbanding dengan tidak dirawat terutamanya OPEFB yang dirawat dengan kaedah gabungan prarawatan. Enzim seluklas 1.5 liter (Novozyme A/S, Denmark) digunakan untuk pensakaridaan EFB yang dirawat dengan pelbagai prarawatan bagi penghasilan gula penurun. Pensakaridaan dilakukan dalam inkubator bergoncang pada suhu 37 °C, 180 rpm. EFB yang telah dikisar, direndam di dalam 0.5 M NaOH dan dipanaskan pada suhu 121 °C, 15 psi memberikan penukaran hasil yang tinggi iaitu 0.53 g gula /g EFB. Ini menunjukkan sembilan kali lebih tinggi berbanding dengan OPEFB yang dirawat secara kimia dan termal iaitu hanya 0.06 g gula/g OPEFB.

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