



Enzymatic Saccharification of Pretreated Solid Palm Oil Mill Effluent and Oil Palm Fruit Fiber

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ABSTRACT

The effectiveness of various chemicals pretreatment (NaOH, HCl, NH₃, HNO₃ and EDTA) on the enzymatic saccharification of solid palm oil mill effluent (POME) and oil palm fruit fibre (OPFF) was investigated. The results showed that NaOH seem to be the most suitable chemical pretreatment for enhancing sugar production and the degree of hydrolysis from saccharification of OPFF. NaOH at a concentration of 2% (w/v) appears to be optimal for alkaline pretreatment of OPFF. However, chemical pretreatment of solid POME using NaOH, NH₃, HNO₃, HCl and EDTA was found to be ineffective in enhancing the degree of hydrolysis and sugar production as compared to chemically untreated solid POME. Autoclaving OPFF at 121°C, 15 psi for 5 minutes improved the degree of hydrolysis up to 2.4 times. However, the degree of hydrolysis was not significantly affected for solid POME under the same conditions.

Keywords: Lignocellulosic materials, cellulase, fermentable sugar, palm oil mill effluent, oil palm fruit fiber

ABBREVIATIONS

OPFF	Oil palm fruit fiber
POME	Palm oil mill effluent
OPEFB	Oil palm empty fruit bunch fiber

INTRODUCTION

As the largest palm oil producer in the world, Malaysian palm oil industries have generated high export revenue because of the favorable price situation. Unfortunately, the production of palm oil from oil palm, *Elaeis guineensis*, also results to concomitant production of wastes such as palm oil mill effluent (POME) and oil palm fruit fiber (OPFF). Utilization of these palm oil wastes to generate energy sources for palm oil mills has been proposed (Ma *et al.*, 1994; Ma *et al.*, 2001). POME has been used as substrate for biohydrogen production using anaerobic contact filter (Vijayaraghavan and Ahmad, 2006) and citric acid production by *Aspergillus niger* (Jamal *et al.*, 2007). POME is also a suitable substrate for biopolymer production due to its high carbon to nitrogen ratio. Volatile fatty acids or organic acids from the anaerobic treatment of POME can be converted to polyhydroxyalkanoate (PHA) by *Rhodobacter sphaeroides* (Pandey, 2006). Industrial enzymes, such as cellulase, can also be produced using oil palm empty fruit bunch fibre as substrate (Umikalsom *et al.*, 1997).

Received : 28 November 2007

Accepted : 10 July 2008

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As lignocellulosic materials, POME and OPFF represent abundant, inexpensive and readily available sources of renewable lignocellulosic materials. These materials appear to be potential carbohydrate sources for fermentable sugars production using cellulolytic enzymes (such as cellulase). However, enzymatic hydrolysis of cellulose is hampered by the low efficiency of cellulolytic enzymes (Chahal, 1991). In general, the cellulolytic enzymes are only able to convert the cellulose portion of the lignocellulose into monomeric sugars. The low cellulose to fermentable sugar (especially glucose) conversion efficiency for lignocellulose is the result of two principle factors, the degree of crystalline nature within the cellulose and the close association between lignin-polysaccharides in plant cell walls (Cowling, 1963; Chahal, 1991). This problem is compounded by the relatively small pore sizes in untreated lignocellulose, which imposes mass transfer limitations on penetration of both microorganisms and cellulolytic enzymes (Detroy *et al.*, 1980). Therefore, structural alteration using practical pretreatment methods is needed in order to tap the cellulosic value contained in POME and OPFF for enzymatic saccharification.

This study was carried out to investigate the effect of different physical and chemical pretreatments on the performance of enzymatic saccharification of solid POME and OPFF.

MATERIALS AND METHODS

Enzymes

The enzyme employed in this study was a commercial enzyme, Celluclast 1.5 L, which is a cellulase from *Trichoderma* sp.. The enzyme in liquid form was supplied by Novo Nordisk. The activity of this cellulase was 47.4 U ml⁻¹, 66.0 U ml⁻¹ and 51.1 U ml⁻¹ for FPase, CMCase and β -glucosidase, respectively. The determination of the enzyme cellulase activity was according to the methods described by Wood and Bhat (1988).

Method of POME and OPFF Pretreatment

POME and OPFF were obtained from Bukit Raja Palm Oil Mill in Klang, Selangor, Malaysia. POME was centrifuged at 3000 rpm for 20 minutes to obtain solid POME and the supernatant discarded. The OPFF was reduced to an average of 2 mm fiber using Waring blender (Braun) followed by grinding using a hammer mill equipped with 2 mm round hole screens (Janke and Kunkel, IKA-Labortechnik, Staufen). The milled OPFF was collected by gravity drop after passing the particles through a 2 mm mesh. The physically treated solid POME and OPFF was preserved in a cold room at 4°C prior to chemical treatments.

Various types of chemicals were used to study the effect of chemical pretreatment on enzymatic saccharification of solid POME and OPFF. Forty grams of either solid POME or OPFF was soaked in 400 ml of different chemical solutions (0.5% w/v) [sodium hydroxide (NaOH), hydrochloric acid (HCl), nitric acid (HNO₃), ammonia solution (NH₃) and ethylenediaminetetra acetic acid (EDTA)] at 30°C for 4 h. In the subsequent experiments, the chemically treated solid POME and OPFF were autoclaved at 121°C, 15 psi for 5 minutes. The effect of different concentrations of NaOH (0.1%, 0.5%, 1.0%, 1.5%, 2.0%, 3%, 4% and 5% w/v) on saccharification of solid POME and OPFF was also carried out using the same pretreatment method as described above. The treated solid

POME and OPFF were filtered and washed with distilled water until no trace amounts of alkali or acid could be detected. The OPFF was dried in an oven at 90°C for 48 h whereas the solid POME was centrifuged at 3000 rpm for 20 minutes to remove the water. Both solid POME and OPFF were preserved in a cold room at 4°C prior to saccharification experiments.

Saccharification Experiment

Enzymatic saccharification of solid POME and OPFF was carried out in a shaking incubator (Certomat, B Braun, Germany) at 40°C and agitated at 200 rpm. For the saccharification process, 4 g of solid POME or OPFF was filled into a 100 ml shake flask, after which 8 ml of cellulase enzyme (Celluclast 1.5L) and 72 ml of 0.05 M sodium acetate buffer (pH 5.0) were added to obtain a 5% (w/v) substrate suspension. Sodium azide (0.02% w/v) was added to the reaction mixture to avoid bacterial or fungal contamination. Samples were collected for analysis at different intervals ranging from 0 to 24 h and 0 to 120 h for solid POME and OPFF, respectively.

Analytical Procedures

Cellulose, hemicellulose and lignin contents in solid POME and OPFF were determined using the gravimetric method as described by Goring and Van Soest (1970). The samples collected from the saccharification experiment, either using solid POME or OPFF, were centrifuged for 10 minutes at 13000 rpm. The supernatant was then used for determination of total reducing sugars and glucose. Total reducing sugars was determined using dinitrosalicylic acid (DNS) according to Miller's (1959) method. Glucose was determined using Sigma Diagnostics Glucose (Trinder) reagent. The degree of hydrolysis was calculated qualitatively according to the method described by Latif *et al.* (1994) using the following equation:

$$\text{Hydrolysis (\%)} = \frac{[\text{reducing sugar (g/l)} \times 0.9 \times 100]}{\text{substrate (g/l)} \times 0.77} \quad (1)$$

RESULTS AND DISCUSSION

Effect of Different Treatments on Chemical Composition of POME) and OPFF

The chemical composition of untreated and treated solid POME and OPFF with different chemicals is summarized in Table 1. Soaking of solid POME and OPEFB in chemical solutions without autoclaving did not produce any significant change in chemical composition as compared to the untreated form of solid POME and OPFF. However, autoclaving the OPFF at 121°C, 15 psi for 5 minutes with NaOH, NH₃ and HNO₃, showed an increase in cellulose content; and a decrease in hemicellulose and lignin content. The increase in cellulose content was dependent on the type of chemical used in the pretreatment. For example, OPFF treated with NaOH gave the highest increase in cellulose content by reducing almost 50% and 25% of the hemicellulose and lignin contents, respectively. Similar results were obtained by Umikalsom *et al.* (1997) when oil palm empty fruit bunch fiber was treated using NaOH and subsequently autoclaved at 121°C, 15 psi for 5 min. The removal of lignin and hemicellulose from OPFF was due to

the swelling effect that caused the disruption of the crystalline regions by NaOH. The swelling of lignocellulose by NaOH involves the breaking of hydrogen bonding and penetration of NaOH into the crystalline regions to solubilize the lignin-hemicellulose complex (Hannes and Leo, 1975). The degree of solubilization was further enhanced by autoclaving the lignocellulose above 100°C. Heat treatment at above 100°C also altered the physical nature of lignin by transforming the lignin into small droplets (Young *et al.*, 1985; Doppelbauer *et al.*, 1987).

About 31% and 17.6% of the hemicellulose content were removed from OPFF after treatment with HCl and EDTA followed by autoclaving, respectively. However, cellulose and lignin content were not significantly different as compared to untreated OPFF. This result is in agreement with those reported by Umikalsom *et al.* (1998) and Detroy *et al.* (1980) where cellulose content in OPEFB and wheat straw treated with 0.5% and 5% EDTA was not significantly increased. Therefore, EDTA and HCl were not suitable to treat OPFF prior to saccharification process.

In comparison to OPFF, chemically treated solid POME exhibited an entirely different response to chemical pretreatment. The cellulose content for all chemical pretreatments, except NaOH, decreased after autoclaving. The highest loss in cellulose content (11.4%) was obtained in solid POME treated with HNO₃, followed by HCl (9.6%) and EDTA (5.0%). However, solid POME treated with NH₃ showed no significant difference after autoclaving. The decrease in cellulose content after autoclaving may be due to the dissolution of cellulose in hot acid during heat treatment. Cellulose consist of a high degree of hydrogen bonding and crystalline structure, rendering it quite unreactive and insoluble in water or common solvents, but it dissolves in acids such as H₂SO₄, H₃PO₄, HCl and HNO₃ at high temperatures (Morris and Sarad, 1990). Apart from that, all of the chemical pretreatments have led to a decrease in hemicellulose and lignin contents. The highest removal of hemicellulose among the chemical pretreatments was given by HCl treated solid POME (51.6%), followed by HNO₃ (35%), NH₃ (31%), NaOH (28%) and EDTA (2.8%). Table 1 shows that the highest lignin removal (24%) was obtained in solid POME treated with HNO₃ as compared to the other chemical pretreatments such as NaOH (14.8%), HCl (14.4%), NH₃ (13.9%) and EDTA (8.5%).

Enzymatic Saccharification of Pretreated Solid POME and OPFF

Effect of Chemical Pretreatment on the Saccharification of Solid POME

The profiles of reducing sugar during saccharification of solid POME pretreated with different chemical solutions (NaOH, NH₃, HCl, HNO₃ and EDTA) are shown in *Fig. 1*. Reducing sugar was produced rapidly during the initial stage of saccharification and the rate declined gradually toward the end of the process. The concentration of reducing sugar reached a maximum value after about 8 h of the saccharification process. Generally, the maximum concentration of reducing sugar produced from saccharification of untreated solid POME (1.53 g l⁻¹) was not significantly different from that obtained in saccharification of solid POME treated with NaOH (1.59 g l⁻¹), NH₃ (1.50 g l⁻¹) and EDTA (1.48 g l⁻¹) (Table 2). However, reducing sugar produced from untreated POME was about 50% higher than that obtained in saccharification of solid POME treated with HCl and HNO₃, where the reducing sugar production was only 0.78 g l⁻¹ and 0.82 g l⁻¹, respectively. The degree of hydrolysis was highest when solid POME was treated with NaOH, followed by untreated solid POME and solid POME treated with NH₃, EDTA, HNO₃ and HCl.

TABLE 1
Chemical composition of untreated and treated solid POME and OPFF with different chemical and physical treatments

Substrate	Treatment	Chemical Composition (%)					
		Cellulose	Hemicellulose	Lignin	Ash	Others	
Solid palm oil mill effluent solid (Solid POME)	Untreated POME Without Autoclaving	39.3 ± 0.51	24.6 ± 0.68	22.2 ± 2.70	1.5 ± 0.20	12.5 ± 2.59	
	NaOH 0.5%	39.2 ± 0.31	24.2 ± 0.51	21.2 ± 1.25	1.1 ± 0.10	14.3 ± 0.95	
	NH ₃ 0.5%	39.0 ± 0.44	23.5 ± 0.61	20.6 ± 0.46	1.4 ± 0.10	16.1 ± 0.69	
	HCl 0.5%	38.5 ± 0.87	23.6 ± 0.56	21.4 ± 0.50	1.4 ± 0.10	15.1 ± 0.84	
	HNO ₃ 0.5%	37.4 ± 0.66	23.9 ± 0.65	20.0 ± 0.81	2.0 ± 0.12	16.7 ± 1.30	
	EDTA 0.5%	37.7 ± 2.25	24.3 ± 0.78	23.0 ± 0.90	2.0 ± 0.13	13.1 ± 2.02	
	Autoclaving						
	NaOH 0.5%	40.2 ± 0.70	17.6 ± 1.08	18.9 ± 0.45	1.2 ± 0.08	22.2 ± 1.08	
	NH ₃ 0.5%	39.3 ± 0.71	16.9 ± 0.85	19.1 ± 0.75	1.3 ± 0.10	23.5 ± 1.45	
	HCl 0.5%	35.5 ± 0.04	14.3 ± 0.80	19.0 ± 0.81	1.3 ± 0.20	29.9 ± 1.04	
	HNO ₃ 0.5%	34.8 ± 0.06	15.9 ± 0.35	16.7 ± 1.31	2.1 ± 0.20	30.6 ± 1.16	
	EDTA 0.5%	37.3 ± 0.53	23.9 ± 1.19	20.3 ± 0.53	2.1 ± 0.27	16.2 ± 0.41	
	Control (autoclave without chemical)	38.7 ± 0.60	24.5 ± 0.68	21.9 ± 2.92	1.4 ± 0.14	13.5 ± 2.38	
Oil palm fruit fiber (OPFF)	Untreated OPFF Without Autoclaving	48.9 ± 0.69	26.0 ± 2.22	15.7 ± 1.12	1.35 ± 0.08	8.1 ± 1.12	
	NaOH 0.5%	50.2 ± 0.36	24.2 ± 0.98	14.2 ± 2.21	1.17 ± 0.28	10.2 ± 2.50	
	NH ₃ 0.5%	48.8 ± 2.02	25.2 ± 1.91	14.5 ± 0.4	1.55 ± 0.07	9.9 ± 2.75	
	HCl 0.5%	49.0 ± 0.56	24.3 ± 1.20	15.3 ± 1.71	1.11 ± 0.16	10.3 ± 1.49	
	HNO ₃ 0.5%	48.9 ± 0.85	24.9 ± 0.91	15.0 ± 1.63	1.59 ± 0.32	9.6 ± 1.60	
	EDTA 0.5%	48.8 ± 0.91	24.2 ± 0.64	15.6 ± 0.35	1.47 ± 0.07	9.9 ± 1.24	
	Autoclaving						
	NaOH 0.5%	53.5 ± 0.93	13.2 ± 0.51	11.8 ± 0.70	1.13 ± 0.25	20.3 ± 1.17	
	NH ₃ 0.5%	50.6 ± 0.70	16.6 ± 1.37	13.2 ± 0.31	1.58 ± 0.09	18.1 ± 1.56	
	HCl 0.5%	49.3 ± 0.38	17.9 ± 1.36	15.0 ± 2.02	1.08 ± 0.14	16.7 ± 2.70	
	HNO ₃ 0.5%	51.3 ± 0.47	14.9 ± 0.91	14.7 ± 1.93	1.46 ± 0.41	17.7 ± 1.68	
	EDTA 0.5%	49.1 ± 1.41	21.4 ± 1.21	15.6 ± 0.46	1.47 ± 0.07	12.4 ± 2.80	
	Control (autoclave without chemical)	47.6 ± 0.46	25.6 ± 1.80	15.2 ± 0.31	1.32 ± 0.03	10.3 ± 1.81	

Values are means of three replicates with ± standard deviation

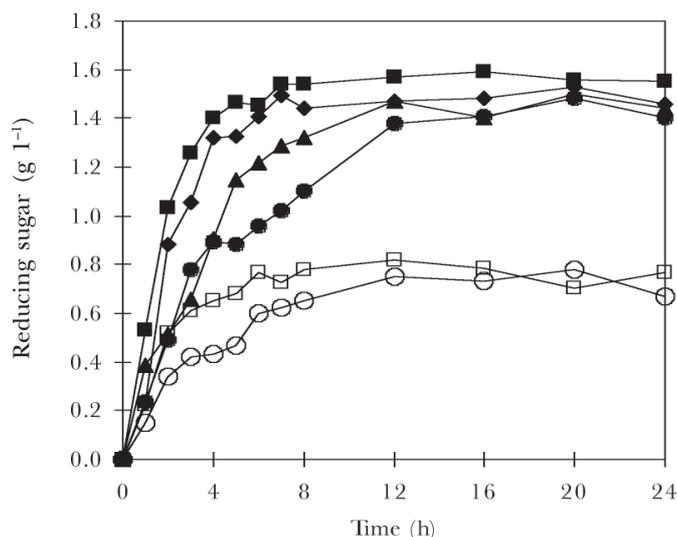


Fig. 1. Patterns of reducing sugar production during saccharification of solid POME pretreated with different chemicals. Symbols represent; (◆) Control (chemically untreated); (■) NaOH (0.5% w/v); (▲) NH₃ (0.5% w/v); (●) EDTA (0.5% w/v); (○) HCl (0.5% w/v); (□) HNO₃ (0.5% w/v)

Among the chemical pretreatment investigated, the solid POME treated with NaOH (1.03 g l⁻¹) resulted in the highest production of glucose, followed by untreated solid POME (1.01 g l⁻¹), NH₃ (0.94 g l⁻¹), EDTA (0.84 g l⁻¹), HNO₃ (0.48 g l⁻¹) and HCl (0.49 g l⁻¹). In addition, glucose yield obtained from untreated solid POME (0.25 g g⁻¹) was comparable to that obtained for Solid POME treated with either NaOH (0.26 g g⁻¹), NH₃ (0.23 g g⁻¹) or EDTA (0.21 g g⁻¹). Reduced production of reducing sugar and glucose during saccharification of solid POME treated with HCl and HNO₃ may be due to excessive loss of soluble cellulose during washing with distilled water after chemical pretreatment. Chahal (1991) reported that the use of dilute mineral, or organic acids in the pretreatment of highly crystalline lignocellulose such as cotton resulted in the formation of less susceptible products due to the removal of more readily accessible cellulose by hot acid, leaving a product of higher degree of order than the starting material. Therefore, HCl and HNO₃ are not good chemicals for pretreatment of solid POME. Additionally, the reducing sugar and glucose production from untreated solid POME was comparable to NaOH treated solid POME and even better than NH₃ and EDTA treated solid POME. Hence, for cost effectiveness, untreated solid POME was used as substrate for enzymatic saccharification in the subsequent experiment.

Effect of Chemical Pretreatment on the Saccharification of OPFF

The time courses of enzymatic saccharification of oil palm fruit fiber (OPFF) treated with NaOH, NH₃, HNO₃, HCl, EDTA and untreated OPFF are shown in Fig. 2. The profile of reducing sugar during saccharification of OPFF was almost similar to the profile of solid POME saccharification, where the reducing sugar was produced rapidly in the first 20 h and declined gradually towards the end of the process. However, chemical pretreatments appear to be more effective in enhancing saccharification of OPFF than in solid POME, as seen from higher values of the reducing sugar production in OPFF as compared to solid POME.

Among the chemical pretreatments investigated, the highest reducing sugar (10.10 g l⁻¹) was obtained from saccharification of OPFF treated with NaOH, which was almost 6-fold higher than that obtained in saccharification of untreated OPEFB (1.74 g l⁻¹) (Table 2). In addition, highest glucose production was obtained from the saccharification of OPFF treated with NaOH (3.56 g l⁻¹), followed by NH₃ (2.67 g l⁻¹), HNO₃ (1.43 g l⁻¹), HCl (1.36 g l⁻¹), EDTA (0.97 g l⁻¹) and untreated OPFF (0.68 g l⁻¹). Furthermore, the highest glucose yield, degree of hydrolysis and productivity was obtained from saccharification of OPFF treated with NaOH, which gave the values of 0.89 g g⁻¹, 88.54% and 0.14 g l⁻¹ h⁻¹, respectively. Similar results were reported by Umikalsom *et al.* (1998), who found that autoclaved oil palm empty fruit bunch (OPEFB) treated with NaOH resulted in the highest rate and degree of hydrolysis as compared to other chemical pretreatments. In addition, OPEFB treated with NaOH could produce hydrolysis yield of up to 85.9%.

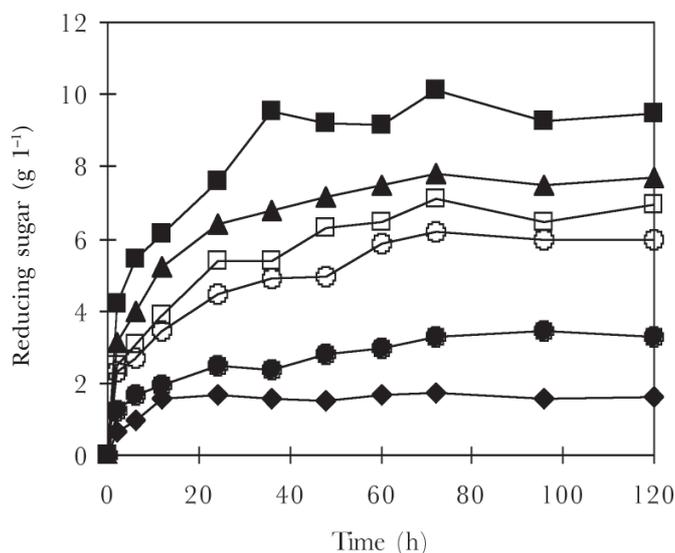


Fig. 2: The patterns of reducing sugar production during saccharification of OPFF pretreated with different chemicals. Symbols represent; (◆) Control (chemically untreated); (■) NaOH (0.5% w/v); (▲) NH₃ (0.5% w/v); (●) EDTA (0.5% w/v); (○) HCl (0.5% w/v); (□) HNO₃ (0.5% w/v)

The treatment of OPFF with NaOH seems to be the best among the chemicals used in this study for the production of reducing sugar and glucose. This may be due to the changes in the structure of OPFF caused by NaOH that led to an increase in its susceptibility to enzymatic hydrolysis. As proposed by Cowling (1963), the influence of structural features on susceptibility of lignocellulosic material to enzyme degradation involved the degree of crystallinity, degree of polymerization, lignin and hemicellulose association, and the unit cell dimensions of the crystalline present. The crystalline in lignocellulosic material can be opened up by swelling agents such as NaOH, anhydrous ammonia and certain amines (Millet *et al.*, 1976). Thus, these intracrystalline swelling agents provide a pathway toward alteration of the cellulose crystalline structure to

TABLE 2
Reducing sugar and glucose production from enzymatic saccharification of solid POME pretreated using different types of chemical

Substrate	Chemical Treatment	Reducing Sugar (g l ⁻¹)	Glucose (g l ⁻¹)	Glucose Yield (g g ⁻¹)	Hydrolysis (%)	Productivity (g reducing sugar l ⁻¹ h ⁻¹)
Solid Palm Oil Mill Effluent (POME)	Control	1.53	1.01	0.25	44.71	0.08
	NaOH 0.5%	1.59	1.03	0.26	46.46	0.10
	NH ₃ 0.5%	1.50	0.94	0.23	43.80	0.07
	HCl 0.5%	0.78	0.49	0.12	22.81	0.04
	HNO ₃ 0.5%	0.82	0.48	0.12	23.96	0.07
	EDTA 0.5%	1.48	0.84	0.21	43.25	0.07
Oil Palm Fruit Fiber (OPFF)	Control	1.74	0.68	0.17	30.51	0.02
	NaOH 0.5%	10.10	3.56	0.89	88.54	0.14
	NH ₃ 0.5%	7.78	2.67	0.67	68.20	0.11
	HCl 0.5%	6.20	1.36	0.34	54.35	0.09
	HNO ₃ 0.5%	6.94	1.43	0.36	62.42	0.10
	EDTA 0.5%	3.46	0.97	0.24	40.44	0.04

enhance enzymatic hydrolysis by reducing the crystalline nature, polymerization and lignin-hemicellulose association (Fox *et al.*, 1978).

The Effect of Autoclaving on Saccharification of Solid POME and OPFF

Fig. 3 shows the effect of autoclaving on the saccharification of chemically pretreated solid POME and OPFF with untreated solid POME and OPFF as control. Generally, the degree of hydrolysis for saccharification of autoclaved chemically treated solid POME did not show any significant difference as compared to non-autoclaved chemically treated solid POME. In addition, the degree of hydrolysis for autoclaved HCl and HNO₃ treated solid POME was even lower than the non-autoclaved solid POME treated with the same chemical. This result indicates that hot acid hydrolyzed the more accessible portion of solid POME during autoclaving leaving the less accessible portion, which affects the performance of enzymatic hydrolysis of solid POME to reducing sugar.

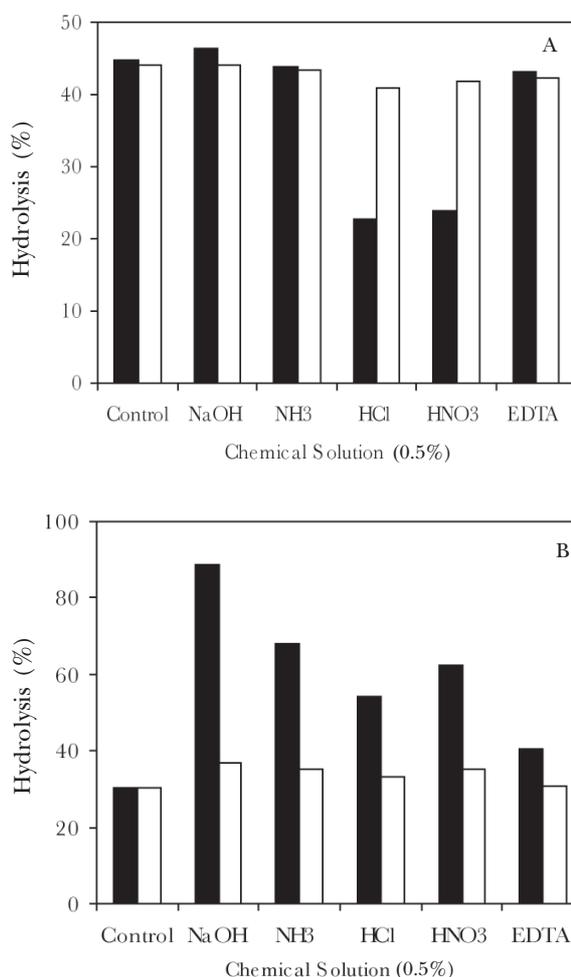


Fig. 3: Effect of autoclaving on the performance of the enzymatic saccharification of chemically treated (A) solid POME and (B) OPFF. Saccharification was carried out at 40 °C, pH 5.0, 200 rpm. (■) autoclaved; (□) non-autoclaved

On the other hand, the increase in hydrolysis for saccharification of OPFF was observed when autoclaved OPFF treated with different chemicals were used as substrate as compared to non-autoclaved OPFF. However, the effect of autoclaving on the degree of hydrolysis was dependent on the type of chemical used for pretreatment. Autoclaving the NaOH treated OPFF gave the highest response to enzymatic saccharification by increasing the degree of hydrolysis up to 2.4 times. The factors that affect autoclaving pretreatment includes residence time, temperature, particle size, moisture content and the addition of an acid catalyst such as sulphuric acid or sulfur dioxide (Sun and Cheng, 2002). However, this was not the case in our findings where the alkaline catalyst (NaOH) was more superior than the acid catalyst. The saccharification of autoclaved oil palm empty fruit bunch (OPEFB) treated with 0.5% NaOH was improved by about 3.5 times, the highest among chemical pretreatments (Umikalsom *et al.*, 1988). Therefore, the treatment of OPFF with NaOH produced the most suitable substrate for enzymatic saccharification as compared to other chemicals (NH_3 , HCl, HNO_3 and EDTA). On the other hand, improvement of enzymatic hydrolysis of wood chips was obtained with steam pretreatment, at very high temperatures, of H_2SO_4 -impregnated materials (Sassner, 2008). In this case, the optimal conditions were obtained at 200°C for 8-10 minutes using 0.5% (w/v) H_2SO_4 .

Effect of Different NaOH Concentrations on the Saccharification of OPFF

The patterns of reducing sugar production during saccharification of OPFF treated with different NaOH concentrations (0.1%, 0.5%, 1.0%, 1.5%, 2.0%, 3.0%, 4.0% and 5.0% w/v) are shown in Fig. 4. In general, reducing sugar linearly increased with increasing NaOH concentration from 0.1% to 1.5% (w/v). However, the production was not significantly different for the OPFF treated with 2% and 5% (w/v) NaOH. The swelling of OPFF caused by NaOH increased with increasing NaOH concentration up to 2% (w/v). Beyond this concentration, the OPFF was fully swelled, as examined under the electron microscope (data not shown) and saturated at 2% w/v NaOH. Hence, NaOH concentration higher than 2% (w/v) did not have any effect on the swelling of OPFF, which makes the cellulose more susceptible to enzyme attack. This is a possible explanation for the increase in reducing sugar production from the saccharification of OPFF treated with concentrated NaOH.

Relationship between Cellulose and Lignin Content on Degree of Hydrolysis from Saccharification of Solid POME and OPFF

The solid POME and OPFF, subjected to different chemical pretreatments (NaOH, HCl, HNO_3 , NH_3 and EDTA), yielded a wide spectrum of cellulose and lignin content (Fig. 5). The degree of hydrolysis from saccharification of solid POME increased slightly and almost linearly with decreasing lignin content from 21.4% to 18.9%. In addition, the degree of hydrolysis for solid POME also increased linearly with increasing cellulose content from 34.8% to 37.4%. However, the degree of hydrolysis only increased slightly from 37.7% to 40.2% for cellulase content of above 38%. This may be due to a collapse of the lignocellulose structure that shrank the availability of surface area for enzymatic attack, which is generated by the removal of the lignin seal (Fan *et al.*, 1981). In the case of OPFF, the degree of hydrolysis increased substantially with decreasing lignin content from 15.7% to 11.8%. On the other hand, the degree of hydrolysis increased but not linearly with increasing cellulose content. Similar observations were reported for enzymatic

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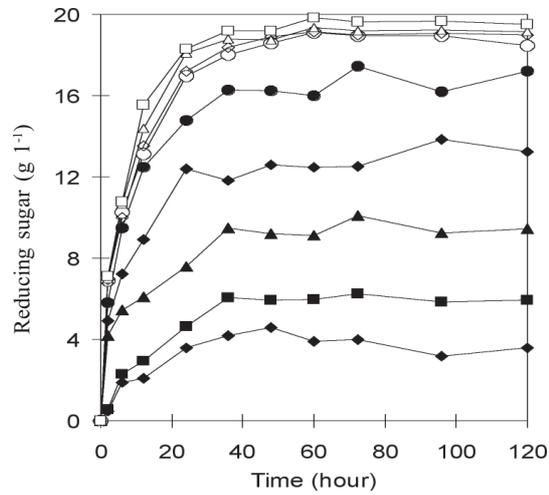


Fig. 4: Patterns of reducing sugar production from enzymatic saccharification of OPFF treated with different NaOH concentration. Symbols represent; (◆) Control; (■) NaOH (0.1% w/v); (▲) NaOH (0.5% w/v); (◆) NaOH (1.0% w/v); (●) NaOH (1.5% w/v); (○) NaOH (2.0% w/v); (◇) NaOH (3.0% w/v); (△) NaOH (4.0% w/v); (□) NaOH (5.0% w/v)

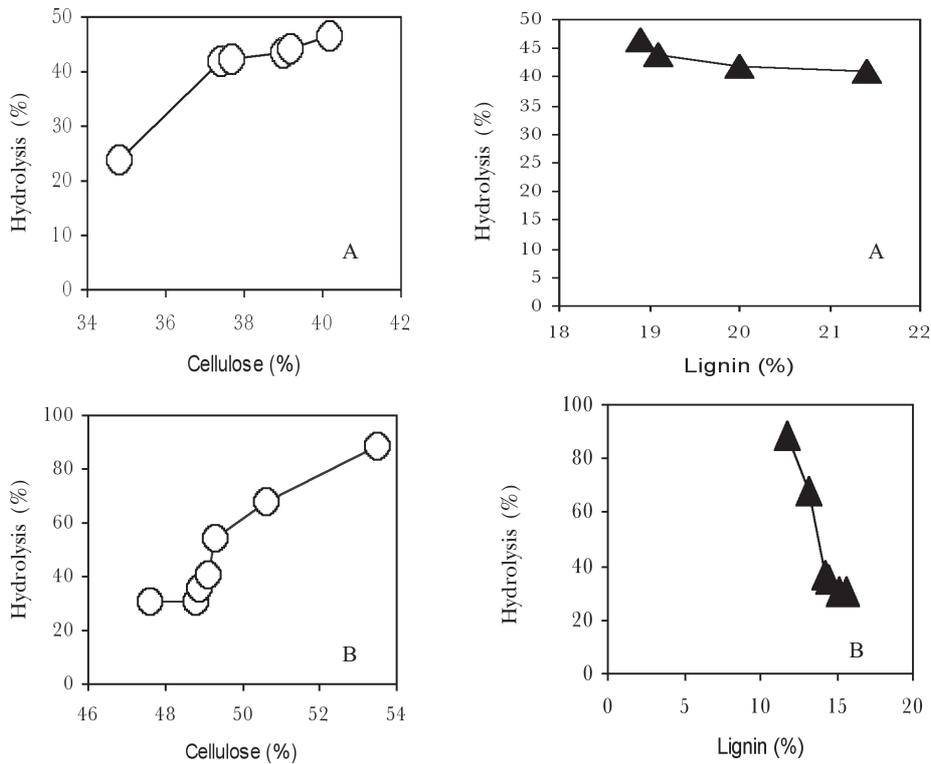


Fig. 5: Relationship between cellulose and lignin contents on the degree of hydrolysis during saccharification of (A) solid POME and (B) OPFF treated with various chemicals (NaOH, HCl, HNO₃, NH₃ and EDTA).

saccharification of oil palm empty fruit bunch treated with various types of chemicals (NaOH, HNO₃, HCl, EDA and EDTA) (Umikalsom *et al.*, 1998).

The recovery of lignin is also important in improving the economy of the process, as lignin can be utilized as a solid fuel (Wingren *et al.*, 2003). During heat and chemical pretreatments part of the lignin undergoes solubilization followed by repolymerization (Sassner *et al.*, 2008). Reactions involving lignin and sugar or sugar degradation products (furfural) also occur (Carrote *et al.*, 1999). The by-products formed in such reaction, known as pseudolignin, contribute to the amount of insoluble lignin in the pretreated materials.

CONCLUSIONS

Results from this study indicate that chemical and physical pretreatments are required for the saccharification of OPFF. Among the chemicals tested (NaOH, HCl, NH₃, HNO₃ and EDTA), the highest saccharification was obtained when OPFF was treated with 2% (w/v). In addition, autoclaving the OPFF at 121°C, 15 psi for 5 minutes also improved the degree of hydrolysis by up to 2.4 times. On the other hand, chemical pretreatment and autoclaving of solid POME did not significantly enhance the saccharification of solid POME as compared to untreated POME. The glucose yield based on the amount of substrate used for optimal enzymatic saccharification of solid POME and OPFF was 0.26 g g⁻¹ and 0.89 g g⁻¹, respectively.

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