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Dilute Acid Pretreatment of Oil Palm Trunk Biomass at High Temperature for Enzymatic Hydrolysis

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Abstract

Old oil palm trunk (OPT) is available in large quantity in Southeast Asia and a potential lignocellulosic biomass resource for bioethanol production. Dilute acid (DA) pretreatment was applied to the old oil palm trunk for enzymatic saccharification. The pretreatment conditions were investigated through a fractional factorial experiment design. The pretreated substrates were analyzed for chemical composition, and their enzymatic digestibility was investigated and compared. The results indicated that the DA pretreatment was able to improve enzymatic hydrolysis by removing hemicelluloses from OPT. Mild pretreatment preserved more hemicellulose and cellulose in pretreated OPT, but severe pretreatment was necessary to achieve satisfactory enzymatic hydrolysis of OPT. For example, the DA pretreatment with 3% H_2SO_4 at 180 ºC for 40 min could achieve an 80% enzymatic hydrolysis.

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Keywords: oil palm trunk; enzymatic hydrolysis; dilute acid pretreatment; cellulosic ethanol

1. Introduction

Lignocellulosic materials such as agricultural residues (wheat straw, corncob, and paddy straw), energy crops (switch grass and fast-grow trees), and forest resources have been recognized as renewable feedstocks for industrial applications to produce bioethanol and other biofuels [1].Oil palm trunk (OPT) is available in large quantity in Southeast Asia and a potential lignocellulosic biomass resource for bioethanol production [2,3]. One of the technologies for converting the biomass into biofuels is the so-called sugar platform. Specifically, the carbohydrates (cellulose and hemicelluloses) in the biomass are

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first hydrolyzed into sugars predominantly by enzymes, and then the sugars are biologically fermented or thermochemically transformed to biofuels. Because of the recalcitrance of the biomass caused by the tough physical structure and the presence of hemicelluloses and lignin surrounding cellulose, the biomass has to be pretreated physically and chemically to remove or reduce the recalcitrance before cellulases can efficiently access and satisfactorily hydrolyze cellulose into glucose. Many methods have been developed and evaluated for biomass pretreatment, such as dilute acid, AFEX, organosolv, steaming, and alkali pretreatment [4,5]. Among them, dilute acid pretreatment is the most investigated method, and it is also an inexpensive and economically feasible process [6-9].This study aims to evaluate the dilute acid pretreatment of OPT for enzymatic hydrolysis. The effects of pretreatment conditions including acid concentration, pretreatment temperature, and reaction time on the dissolution of biomass components (cellulose, hemicelluloses, and lignin), sugar yield, formation of fermentation inhibitors, and enzymatic digestibility of the pretreated OPT will be investigated.

2. Methodology

2.1. Raw material

Twenty-five year-old of oil palm (*Elaeis guineensis*) trunks (OPT) were collected from an oil palm plantation in Phangnga Province of Southern Thailand. The length of OPT was in the range of 12-15 m, with a diameter of 35-80 cm. The top part of the OPT was cut at the length of 3 m and chipped. Then, the raw material was air-dried at room temperature to equilibrium moisture content to about 10%, milled using a laboratory hammer mill to 0.5-1 mm, homogenized in a single lot and stored until the usage. The composition of the OPT were 12.4% moisture, 17.0% acid-insoluble lignin, 4.4% acid-soluble lignin, 6.7% extractives, 2.9% ash, 38.1% glucose, 23.1% xylose, 0.7% galactose, and 0.6% arabinose.

Table 1. Fractional factorial experimental design and results of OPT dilute acid pretreatment.

Run	Pretreatment condition				Solid yield (%)	Glu-Rec-Pre (%)	Glu-Rec-Enz (%)	Solid composition (%)			
	SA (%)	Temp (ºC)	Time (min)	CS				Glu	Xyl	AIL	ASL
1	1	160	20	2.13	72.2	82.7	18.2	43.6	13.1	21.4	3.7
2	3	170	20	2.87	64.0	92.9	46.5	55.3	1.7	27.2	3.0
3	2	180	20	3.00	56.5	82.4	44.5	55.6	0.8	28.4	2.8
4	3	160	30	2.75	62.6	91.3	45.7	55.6	1.7	26.5	3.2
5	2	170	30	2.88	59.7	84.5	41.4	54.0	2.6	25.9	3.2
6	1	180	30	2.89	64.8	81.1	41.4	47.7	5.4	24.1	4.1
7	2	160	40	2.71	60.3	89.0	49.8	56.3	2.8	24.1	3.5
8	1	170	40	2.72	59.2	85.8	44.6	55.3	2.6	23.9	3.5
9	3	180	40	3.47	46.8	64.3	50.8	52.3	ND	37.3	2.6

Note: (1). SA – sulfuric acid; Glu – glucose; Xyl – xylose; AIL – acid-insoluble lignin; ASL – acid-soluble lignin; and ND – not detected.(2). CS – combined severity, $CS = \log(R_0) - pH, R_0 = \int_0^t \left[\frac{(T_H - T_R)}{14.75} \right] dt.$ t: reaction time (minutes), T_H: reaction temperature (°C), T_R: reference temperature (100 °C), pH: pH of pretreatment liquid. (3). Glu-Rec-Pre – glucose recovery after pretreatment, the percentage of glucose retained in pretreated OPT to the original glucose in unpretreated OPT. (4). Glu-Rec-Enz – overall glucose recovery after enzymatic hydrolysis based on original glucose in OPT.

2.2. Dilute acid pretreatment

Dilute acid pretreatment was performed at laboratory scale with a microwave reactor manufactured by CEM Corporation (Model MARS, Matthews, NC, USA) with 6 reaction vessels. Each vessel has a total

volume of 50 mL. The amount of dry feedstock loaded was 10 g, and dilute acid was added at 1:5 (w/v) solid/liquid ratio. Both the acid and raw material were initially at room temperature. A fractional factorial design was used for the pretreatment experiments, and the pretreatment conditions are summarized in Table 1. The pretreatment temperature was changed within the range of 160-180 ºC; reaction time was from 20 to 40 min; and initial H_2SO_4 concentration was from 1% to 3% (w/w). When the pretreatment was finished, the pretreatment liquor was separated from solid by filtration. The filtrate was analyzed by high performance liquid chromatography (HPLC) to determine the concentration of glucose, xylose, arabinose, galactose, formic acid, acetic acid, levulinic acid, furfural, and hydroxylmethylfurfural in the hydrolysate. The mass and moisture content of the solid fraction were measured to determine solid yield, and the solid was stored in a fridge for composition analysis and enzymatic hydrolysis.

2.3. Enzymatic hydrolysis (cellulose-to-glucose conversion)

Commercial enzymes, cellulase and β-glucosidase, were generously provided by Novozymes North America (Franklinton, NC, USA). The hydrolysis was carried out at 50 °C on a shaking incubator at 200 rpm. Substrate equivalent to 0.8 g cellulose was loaded into a 50-mL falcon tube with 20 mL of 0.05 M sodium acetate buffer (pH 4.8). Approximately 20 μL of 5 % tetracycline chloride was used to control the growth of microorganisms and prevent consumption of liberated sugars. Cellulase (15 FPU (Filter Paper Unit) per gram cellulose) and β-glucosidase (30CBU (Cellobiase Unit) per gram cellulose) were loaded into the tube. Hydrolysate was sampled periodically and subjected to glucose analysis [10].

2.4. Analytical methods

Sugar analysis for glucose, xylose, arabinose, mannose, and galactose was conducted using a Dionex HPLC system (ICS-3000) equipped with an integrated amperometry detector and Carbopac™ PA1 guard and analytical columns at 20 ºC [11]. Fermentation inhibitors generated in the pretreatment including acetic acid, formic acid, furfural, levulinic acid, and hydroxymethylfurural were analyzed using the Dionex ICS-3000 equipped with a Supelcogel C-610H column at temperature 30 ºC and a UV detector at 210 nm. Eluent was 0.1% phosphoric acid at a flow rate of 0.7 mL/min [11]. Lignin (acid-insoluble and acid-soluble lignin) was analyzed according to the NREL protocol [11]. All data reported were the average of three determinations.

3. Results

3.1. Substrate and glucose yields

It was found that severe pretreatment conditions, such as elevated temperature, longer pretreatment time and higher acid concentration, reduced the yield of substrate (pretreated OPT), as shown in Table 1. Combined severity (CS, as defined underneath Table 1) was used to compare different pretreatments, which is a combined factor of pretreatment temperature, time, and acid loading. The results indicated that harsh conditions enhanced the dissolution of biomass components during the pretreatment. For example, the maximum substrate yield was approximately 72.2%, achieved when acid concentration, temperature and time were 1%, 160 ºC and 20 min, respectively, which was the mildest condition investigated with the lowest CS of 2.13 (Run 1 in Table 1). On the other hand, the lowest substrate yield (46.8%) was achieved when acid concentration, temperature and time were 3%, 180 ºC and 40 min, respectively, which represented the severest condition with the highest CS (3.47) investigated (Run 9 in Table 1).

The composition of pretreated OPT substrates in Table 1 indicated that hemicelluloses were preferably removed during the pretreatment. No arabinose and galactose were detected in the substrates, and xylose content was dramatically reduced, in particular at severer conditions (higher CS), compared to unpretreated OPT. However, cellulose was selectively retained in the pretreated OPT substrates, observed

as increased glucose content, compared to unpretreated OPT. The overall cellulose (glucose) recovery during the pretreatment (Glu-Rec-Pre in Table 1) was ~80-90% in most of pretreatment runs except for Run 9 that had only ~64% glucose recovery because of the highest CS, suggesting that extremely harsh conditions could cause excessive loss of cellulose. Since dilute acid is unable to delignify biomass, lignin was enriched in the substrates after the pretreatment when most of hemicelluloses and part of cellulose were dissolved. The lignin content was positively correlated to the CS because severer pretreatment removed more hemicelluloses and cellulose and concentrated lignin in the pretreated substrate.

3.2. Sugars and fermentation inhibitors in pretreatment liquor

Most of the hemicelluloses and small portion of cellulose were dissolved through acid-catalyzed hydrolysis during the pretreatment and presented in the pretreatment liquor in the form of monomeric sugars, which are potential fermentable sugars for ethanol production. In general, severer pretreatment (higher CS) would dissolve more hemicelluloses and cellulose. Severer condition would also cause degradation of the sugars to furfural (FF, from pentoses) and hydroxymethylfurfural (HMF, from hexoses) through dehydration. HMF could be further decomposed to levulinic acid (LA) and formic acid (FA) through rehydration [12-14]. All of these were observed during the acid pretreatment of OPT. As shown in Table 2, the concentrations of the sugars in the pretreatment liquor increased first and then decreased with the increased CS because of the further degradation of the sugars, which was supported by the fact that more FF and HMF were detected in the liquor at higher CS, for example Run 9 (CS = 3.47). The sugar degradation products including FF, HMF, LA, and FA not only reduced the yield of fermentable sugars in the pretreatment liquor but also served as fermentation inhibitors. They have to be removed or reduced by detoxification before the sugars can be fermented into ethanol or other products. The lignin degradation products (detected as acid-soluble lignin) and acetic acid from hemicelluloses are additional inhibitors to fermentation.

Table 2. Yields of sugars, soluble lignin, and sugar degradation products in pretreatment liquor.

Run	CS	Yield (%)									
		Ara	Gal	Glu	Xyl	ASL	FA	AA	LA	HMF	FF
1	2.13	0.4	0.3	0.2	1.3	0.6	0.55	0.72	0.70	3.70	ND
2	2.87	0.4	0.4	0.5	5.6	1.3	1.27	1.21	0.50	0.96	0.04
3	3.00	0.3	0.3	0.5	4.6	1.2	1.24	1.63	0.47	1.77	0.06
4	2.75	0.4	0.3	0.4	5.5	1.1	1.69	2.05	0.62	1.57	0.02
5	2.88	0.3	0.3	0.4	3.8	0.9	1.12	1.53	0.55	1.42	0.01
6	2.89	0.2	0.3	0.4	2.5	0.8	1.33	2.83	0.58	1.77	0.06
7	2.71	0.3	0.3	0.4	3.7	0.8	1.35	1.77	0.46	0.93	0.01
8	2.72	0.2	0.3	0.4	2.8	1.2	1.28	2.72	0.67	1.52	0.04
9	3.47	0.2	0.2	2.3	3.5	2.8	2.95	6.06	2.53	2.64	0.78

Note: CS – combined severity; Ara – arabinose; Gal – galactose; Glu – glucose; Xyl – xylose; ASL – acid soluble lignin; FA – formic acid; AA – acetic acid; LA – levulinic acid; HMF – hydroxymethylfurfural; FF – furfural, and ND – not detected.

3.3. Enzymatic hydrolysis of acid pretreated OPT substrates

The substrate characteristics that affect enzymatic hydrolysis of cellulose include hemicellulose content, lignin structure, distribution and content, cellulose crystallinity and degree of polymerization, and surface area, pore size and particle size of the substrate [11,15,16].

After the majority of the hemicelluloses in OPT were removed during the pretreatment, as discussed above, the remaining solid (mainly cellulose and lignin) was more porous and could be more easily

hydrolyzed by cellulases into glucose. In order to evaluate the effects of the pretreatment process and conditions on subsequent enzymatic hydrolysis, the solids after the acid pretreatment were hydrolyzed by the enzymes consisting of cellulase at loading of15 FPU/g cellulose and β-glucosidase at loading of 30 CBU/g cellulose for 72 h.

The 72-h enzymatic hydrolysis profiles of dilute acid pretreated OPT substrates are shown in Figure1.The substrate #1 pretreated at 1% H_2SO_4, 160 ºC, and 20 min showed the lowest cellulose-to-glucose conversion yield of 22% at 72 hour, while the substrate #9 pretreated at 3% H_2SO_4, 180 ºC, and 40 min gave the highest cellulose-to-glucose conversion yield of 79%.The substrates pretreated at other conditions had very similar enzymatic digestibility, and their 72-hour cellulose-to-glucose conversion yields fell to 50-56%. The poor enzymatic digestibility of substrate #1 was attributed to its mild pretreatment that was not severe enough to remove sufficient hemicelluloses. As shown in Table 1, after the pretreatment, substrate #1 still had ~13% hemicelluloses, which was the major cause of the poor digestibility. In contrast, all hemicelluloses were removed from substrate #9 that was pretreated at the severest condition, which was the predominant reason why this substrate had the best enzymatic digestibility. In addition, the high acid concentration, high temperature and long pretreatment time might have prehydrolyzed (depolymerized) cellulose in substrate #9 to certain extent, which certainly enhanced the enzymatic hydrolysis of cellulose. The results suggested that severe condition was necessary for OPT to get a better enzymatic hydrolysis.

The overall glucose recovery yield after pretreatment and subsequent enzymatic hydrolysis was calculated and listed in Table 1 (Glu-Rec-Enz). Run 9 gave the highest overall glucose yield, although it had the lowest recovery glucose yield in pretreatment (Glu-Rec-Pre). The results suggested that in order to get satisfactory overall glucose yield, sufficient pretreatment is crucial. Although mild pretreatment could retain more cellulose in substrate (Table 1), the cellulose was not easily accessible and digestible to cellulases because mild pretreatment was unable to satisfactorily remove biomass recalcitrance.

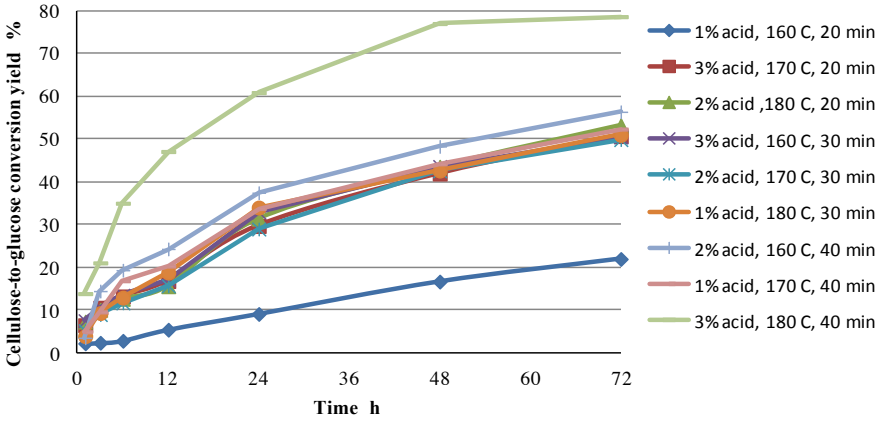

Fig.1. Cellulose-to-glucose conversion yield during the enzymatic hydrolysis of dilute acid pretreated OPT at different conditions.

4. Conclusions

Dilute acid pretreatment of OPT at varying conditions (acid concentration, temperature, and time) indicated that the pretreatment could effectively remove hemicelluloses from OPT, which improved enzymatic digestibility of OPT. Milder pretreatment preserved more hemicelluloses and cellulose in the pretreated OPT, but enzymatic hydrolysability was unsatisfactory because the biomass recalcitrance was not sufficiently removed. Severer pretreatment suffered from low sugar recovery, but the resultant substrate had much better enzymatic digestibility. The results suggested that the pretreatment with 3%

H_2SO_4 at 180 ºC for 40 min could not only achieve an ~80% enzymatic hydrolysis and but also the highest overall glucose recovery, although the glucose recovery in the pretreatment was low.

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