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# Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose for Enzymatic Hydrolysis of Oil Palm trunk

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**Abstract**

Oil palm trunk (OPT) an abundant agriculture waste was evaluated as an alternative lignocellulosic biomass resource for bioethanol production. Sulfite-based SPORL pretreatment (Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose) was applied to the OPT to enhance its enzymatic saccharification. The pretreatment conditions were optimized through a designed experiment within the ranges of temperature 170–190ºC, time for 30 min, 2–6% sulfite, and initial $H_2SO_4$ concentrations 3–7% (w/w). The overall saccharides (hexoses and pentoses) recovery of SPORL pretreated was 62.5%. SPORL pretreatment removed 38.8% lignin from the OPT as lignosulfonate in the liquor with great potential for co-product development, and 93.9% xylose was dissolved, which were the main reasons why the SPORL pretreatment could enhance the enzymatic hydrolysis of the OPT. The results indicated that over 92% of the cellulose in the pretreated OPT with 7% $H_2SO_4$ and 6% $Na_2SO_3$ at 190ºC was enzymatically hydrolyzed to glucose within 48 h with a cellulase loading of 15 FPU/g cellulose, and overall glucose yield was 66%.


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*Keywords:* Oil palm trunk; SPORL; lignocellulose; bioethanol; enzymatic hydrolysis

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## 1. Introduction

Oil palm trees generally start bearing oil-containing fruits 3 years after planting, but oil productivity starts decreasing after 20-25 years. Therefore, it is necessary to cut the old oil palm trunk (OPT) and replant new seedlings at plantation sites to maintain the oil productivity [1]. The OPT of the palm trees felled in the harvested area is an abundant biomass. The total volume of the OPT is about 1.44 million tons/year, equivalent to crude oil potentials of 258.12 ktoe/year or electrical energy 604 million kW-h/year, representing an installed electrical power of about 71.90 MW (at 20% efficient power plant operation 350 days/year). However, the OPT has not been successfully used for fuel production and other uses [2].

The OPT biomass consists of cellulose, hemicelluloses, and lignin. Cellulose and hemicelluloses can be converted into fermentable sugars and subsequently to fuel ethanol. In order to improve the accessibility of cellulolytic enzymes to the OPT, the OPT structure must be broken down through pretreatment. The pretreatment stage is therefore critical to improve the recovery of fermentable sugars from the biomass. In our previous study [3], dilute acid method was investigated to pretreat the OPT. The pretreatment could significantly improve the enzymatic hydrolysis of the OPT, but the glucose recovery was not satisfactory due to the acid-induced sugar degradation, which generated significant amount of furfural and hydroxymethylfurfural that are fermentation inhibitors.

Sulfite pretreatment to overcome recalcitrance of lignocelluloses (SPORL) is a sulfite-based pretreatment method and has been demonstrated to be a very efficient method for pretreating woody biomass for ethanol production [4-5]. The goal of sulfite pretreatment is to remove lignin partially without concurrent loss and degradation of hemicelluloses and cellulose, leading to accessible substrates by cellulase with high yield. In addition, sulfonation of lignin increases the hydrophilicity of lignin, which will reduce the negative impact of lignin on the enzymatic hydrolysis. In this study, a fractional factorial design method was used to optimize the sulfite pretreatment of oil palm trunk, in respect to sulfite charge (2–6%, based on dry OPT), sulfuric acid (3–5%, based on dry OPT), and temperature (170–190 °C) for maximizing glucose production in consequent enzymatic hydrolysis process.

## 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 [3]. Then, the raw material was air-dried at room temperature to equilibrium moisture content of about 10%, milled using a laboratory hammer mill to 0.5-1 mm, homogenized in a single lot and stored until the use.

### *2.2 Experimental design*

OPT was pretreated at 9 different sets of operational conditions according to a fractional factorial design [3**(3-1) fractional factorial design] including 3 factors, as shown in Table 1. Pretreatment experiments were performed in random order. The recovery of cellulose and hemicelluloses in the pretreated solids, and the concentration of glucose and hemicellulosic sugars in the liquid fractions released from pretreatment were determined as responses. In addition, degradation of glucose and other sugars as a consequence of pretreatment was also evaluated. The experimental data were analyzed using the STATISTICA software 10.0.

Table 1. Factors and levels of the fractional factorial design

| Factors | Levels | | |
|---|---|---|---|
| $H_2SO_4$ (%) | 3 | 5 | 7 |
| $Na_2SO_3$ (%) | 2 | 4 | 6 |
| Temperature (ºC) | 170 | 180 | 190 |

*2.3 SOPRL pretreatment*

SPORL pretreatment was performed at laboratory scale with a microwave reactor. The reaction vessel has a total volume of 50 mL. The amount of dry feedstock loaded was 10 g, and pretreatment liquor was added at 1:5 (w/v) solid/liquid ratio. Both the liquor and raw material were initially at room temperature. The other conditions of pretreatment are summarized in Table 2. The pretreatment conditions were varied with temperatures 170–190 ºC, reaction time 30 min, sulfite loading 2–6%, and initial $H_2SO_4$ concentrations 3%– 5% (w/w). When the pretreatment was finished, the pretreated liquor was separated by filtration. The liquid fraction was analyzed by high-pressure liquid chromatography (HPLC) to determine the concentration of glucose, xylose, arabinose, galactose, formic acid, acetic acid, levulinic acid furfural, and HMF in the hydrolysate [4]. The solid fraction was analyzed for moisture and composition, and then stored in a freezer for the next step.

Table 2. Fractional factorial design of SPORL pretreatment experiments

| Run | Code | $H_2SO_4$ (%) | $Na_2SO_3$ (%) | Temperature (ºC) | Initial liquor (pH) |
|---|---|---|---|---|---|
| 1 | 000 | 3 | 2 | 170 | 2.26 ± 0.01 |
| 2 | 210 | 7 | 4 | 170 | 1.87 ± 0.04 |
| 3 | 120 | 5 | 6 | 170 | 2.60 ± 0.08 |
| 4 | 201 | 7 | 2 | 180 | 1.69 ± 0.01 |
| 5 | 111 | 5 | 4 | 180 | 2.12 ± 0.01 |
| 6 | 021 | 3 | 6 | 180 | 3.68 ± 0.07 |
| 7 | 102 | 5 | 2 | 190 | 1.85 ± 0.06 |
| 8 | 012 | 3 | 4 | 190 | 2.79 ± 0.01 |
| 9 | 222 | 7 | 6 | 190 | 2.27 ± 0.01 |

*2.4 Enzymatic hydrolysis (cellulose-to-glucose conversion)*

Cellulase and β-glucosidase produced by Novozymes were purchased from Sigma. The enzymatic hydrolysis of pretreated OPT 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 tube with 20 mL of 0.05 M sodium acetate buffer (pH 4.8). Cellulase (15 FPU, Filter Paper Units, per gram cellulose) and β-glucosidase (30 CBU, Cellobiase Units, per gram cellulose) were loaded into the tube. Hydrolysates were sampled periodically and subjected to glucose analysis [6].

*2.5 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 [4]. 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 [4]. Lignin (acid-insoluble and acid-soluble lignin) was analyzed according to the NREL protocol [4]. All data reported were the average of three determinations.

## 3. Results and Discussion

*3.1 Substrate and glucose yields*

The original OPT was composed of 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. Chemical composition of the SPORL pretreated OPT under different conditions is presented in Table 3. In general, more sulfuric acid, less sulfite, and high temperature promoted the dissolution of hemicelluloses and partial hydrolysis of cellulose; higher sulfite loading enhanced the removal (dissolution) of lignin through sulfonation, and meanwhile protected hemicelluloses and cellulose from excessive hydrolysis because sulfite buffered pH at slightly higher level.

Table 3. Glucose, xylose and lignin of pretreated oil palm trunk substrates under varied conditions

| Run | $H_2SO_4$ (%) | $Na_2SO_3$ (%) | Temp. (ºC) | Initial liquor (pH) | Substrate yield (%) | Glucose (%) | Xylose (%) | Lignin (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 2 | 170 | 2.26 | 53.2 | 63.1 | 7.1 | 23.2 |
| 2 | 7 | 4 | 170 | 1.87 | 44.4 | 68.8 | 4.9 | 30.1 |
| 3 | 5 | 6 | 170 | 2.6 | 57.5 | 62.2 | 11.6 | 19.9 |
| 4 | 7 | 2 | 180 | 1.69 | 41.6 | 44.2 | 0.0 | 56.9 |
| 5 | 5 | 4 | 180 | 2.12 | 53.5 | 64.6 | 5.5 | 26.6 |
| 6 | 3 | 6 | 180 | 3.68 | 67.0 | 56.5 | 14.0 | 20.2 |
| 7 | 5 | 2 | 190 | 1.85 | 44.6 | 62.2 | 3.8 | 35.5 |
| 8 | 3 | 4 | 190 | 2.79 | 57.7 | 64.2 | 7.5 | 24.2 |
| 9 | 7 | 6 | 190 | 2.27 | 47.7 | 47.0 | 2.9 | 27.5 |

*3.2 Sugars and fermentation inhibitors in pretreatment liquor*

The hemicelluloses and small portion of cellulose were dissolved through SPORL pretreatment and presented in the pretreatment liquor in the form of monomeric sugars, which are potential fermentable sugars for ethanol production. 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 formic acid (FA) through rehydration [7-8]. All of these were observed during the SPORL pretreatment of OPT. As shown in Table 4, the sugar degradation products including FF, HMF, and FA not only reduced the yield of fermentable sugars (pentoses and hexoses) in the pretreatment liquor but also served as fermentation inhibitors (furfural and HMF).

Table 4. Yields of sugars, soluble lignin, and sugar degradation products in pretreatment liquor

| Run | Yield (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ara | Gal | Glu | Xyl | ASL | FA | AA | HMF | FF |
| 1 | 0.53 | 0.32 | 0.77 | 3.65 | 2.39 | 1.59 | 3.64 | 2.87 | 0.04 |
| 2 | 0.63 | 0.38 | 1.81 | 5.54 | 3.40 | 2.31 | 6.94 | 2.71 | 0.12 |
| 3 | 0.48 | 0.20 | 0.37 | 1.09 | 2.83 | 1.10 | 0.87 | 3.93 | 0.09 |
| 4 | 0.33 | 0.23 | 7.81 | 2.27 | 4.80 | 3.22 | 11.05 | 2.89 | 0.91 |
| 5 | 0.44 | 0.29 | 0.90 | 3.59 | 3.47 | 2.17 | 8.53 | 2.92 | 0.12 |
| 6 | 0.25 | 0.07 | 0.06 | 0.14 | 2.40 | 0.64 | 0.19 | 1.75 | 0.06 |
| 7 | 0.62 | 0.35 | 3.70 | 4.15 | 4.03 | 3.14 | 12.46 | 2.92 | 0.64 |
| 8 | 0.27 | 0.14 | 0.43 | 1.21 | 2.67 | 1.66 | 6.20 | 4.49 | 0.04 |
| 9 | 0.34 | 0.23 | 1.66 | 2.59 | 6.20 | 3.10 | 9.80 | 2.93 | 0.92 |

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

*3.3 Mass balance of SPORL pretreated OPT*

Establishing mass balance for the pretreatment and following hydrolysis steps is an important way to evaluate a pretreatment method [9-11]. However, it is not easy to close the mass balance accurately because of the complexity of the degradation products [7, 12]. As shown in Fig. 1., the feedstock OPT was separated into two fractions after the pretreatments, solid substrate and a liquid stream (spent pretreatment liquor) containing dissolved sugars, lignin, and sugar degradation products. From 100 g of oven-dry OPT (containing 38.1 g glucose, 23.1 g xylose, 0.6 g arabinose, 0.7 g galactose, and 21.4 g lignin), 47.7 g solid substrate (containing 22.4 g glucose, 1.4 g xylose, and 13.1 g lignin) and spent pretreatment liquor (containing 1.7 g glucose, 26.5 g xylose, and small amount of arabinose and galactose) were produced. Specifically, 38.8% lignin was removed from the OPT as lignosulfonate in the liquor, and 93.9% xylose was dissolved, which were the main reasons why the SPORL pretreatment could enhance the enzymatic hydrolysis of the OPT.

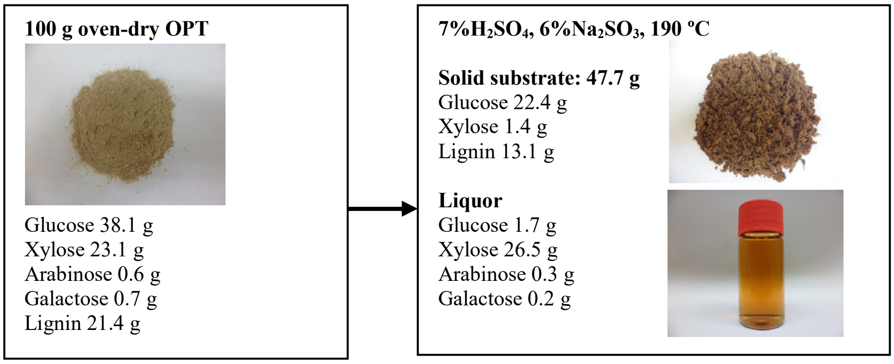


Fig. 1. Mass balance of SPORL pretreatment of OPT with 7% $H_2SO_4$ and 6%$Na_2SO_3$ at 190 ºC for 30 min

*3.4 Enzymatic hydrolysis of SPORL pretreated OPT substrates*

Generally removing hemicellulose and lignin, swelling cellulose to destroy crystallinity, pre-hydrolyzing cellulose to shorten chain length (increasing the number of chain ends for enzymes to attack), and increasing surface area or decreasing particle size are favorable for enzymatic digestibility of cellulosic substrates [4]. After the hemicelluloses in OPT were dissolved, the remaining solid (mainly cellulose and lignin) became porous and could easily be hydrolyzed by cellulases into glucose. In order to evaluate the effects of the pretreatment process on consequent enzymatic hydrolysis, the solids after SPORL pretreatment were hydrolyzed by cellulase of 15 FPU/g cellulose and β-glucosidase of 30 CBU/g cellulose for 48 h. The cellulose-to-glucose conversion and overall glucose yield of three SPORL pretreated OPT samples under different conditions are shown in Fig. 2. The results indicate that over 92% of the cellulose in the pretreated OPT with 7% $H_2SO_4$ and 6% $Na_2SO_3$ at 190ºC was enzymatically hydrolyzed to glucose within 48 h and overall glucose yield was 66%.

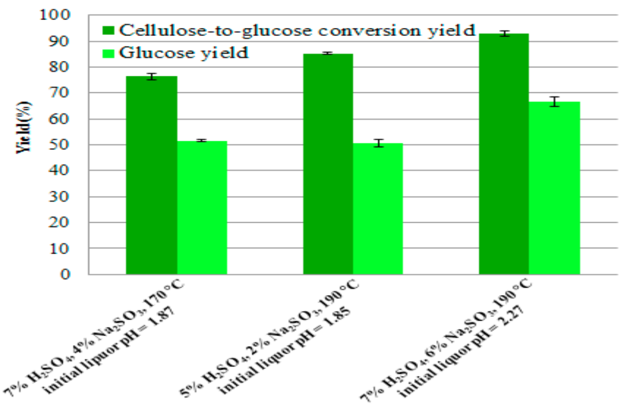


Fig. 2. Cellulose-to-glucose conversion and overall glucose yield through 48 h enzymatic hydrolysis of SPORL pretreated oil palm trunk under different pretreatment conditions

## 4. Conclusions

SPORL pretreatment significantly removed the recalcitrance of oil palm trunk and allowed nearly complete enzymatic hydrolysis (>90%) within 48 h with a cellulase loading of 15 FPU/g cellulose. The overall saccharides (hexoses and pentoses) recovery of SPORL pretreated was 62.5%. In addition, the SPORL pretreatment removed 38.8% lignin from the OPT as lignosulfonate in the liquor with great potential for co-product development, and 93.9% xylose was dissolved, which were the main reasons why the SPORL pretreatment could enhance the enzymatic hydrolysis of the OPT.


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