

## Optimization of organosolv pretreatment of starch waste from sugar palm trunk (*Arenga pinnata*) for the production of reducing sugar

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### Abstract

*Arenga pinnata* is rich in hemicellulose (27.95 %) and lignin (36.98 %). In this study, it was processed in a three-step process to produce reducing sugar; lignocellulose pretreatment, optimization and enzymatic hydrolysis. A combination of acid and organosolv pretreatment was expected to remove a significant amount of lignin from the biomass, but not dissolve much hemicellulose. Response surface methodology (RSM) was employed to determine optimum operational conditions (ethanol concentration, pretreatment time and temperature) for the organosolv pretreatment, which minimized inhibition by lignin in the enzymatic hydrolysis process. Lignin content was analyzed using gravimetric method. Reducing sugar was analyzed using the 3,5-dinitrosalicylic acid (DNS method) and validated by high-performance liquid chromatography (HPLC). From the RSM results, it can be concluded that all the variables studied were significant ( $p < 0.05$ ), apart from the interaction between pretreatment time and temperature ( $p > 0.05$ ), which had an  $R^2$  of 99.38 %. The optimum conditions for organosolv pretreatment were obtained at 30.35% (v/v) ethanol concentration, 107 °C, and 33 minutes pretreatment time. The enzymatic hydrolysis process was conducted with different combinations of enzymes, and with Tween 80 as a surfactant. The best yield of reducing sugar was 20.28 % by using the combination of cellulase-xylanase enzymes with Tween 80, while the combination of enzymes without Tween 80 resulted in a yield of 18.15 %.

**Keywords:** Enzymatic hydrolysis, Organosolv pretreatment, Response surface methodology, Sugar palm starch waste, Tween 80

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## Introduction

Starch waste from sugar palm trunks (SWSPT) is obtained from 96 % solid waste in the production of sugar palm flour. SWSPT has a high content of cellulose and hemicellulose which are potential as a raw material in the production of reducing sugar,

which can then be converted into alcohol-based fuel by fermentation, or gas fuel by gasification (Liang et al., 2017). The production of sugar palm trees in 2015 in Indonesia was 11398.70 tons from an area of 2041.72 ha (BPS Tasikmalaya, 2015). In Indonesia, and particularly on the island of Java, palm trees grow in damp, heterogeneous forests, so the recovery of



waste from palm flour production is somewhat more difficult than that of other agricultural wastes. However, it has been found that lignin content in sugar palm waste can act as an inhibitor, with fiber-swelling properties capable of inhibiting the accessibility of enzyme into the substrate. Therefore, a delignification process is necessary.

There are five commonly used pretreatment methods for this process: acid, hydrothermal, mild alkaline, oxidative and organosolv (Jönsson and Martín, 2016). The acid-based method is capable of reducing hemicellulose content with the advantage of low production costs, but it also causes corrosion problems. The hydrothermal method is capable of dissolving hemicelluloses and releasing sugar from them, does not require many catalysts and does not cause significant corrosion problems; however, this type of pretreatment has to be carried out at high pressure and so the equipment required is expensive. The mild alkaline method is capable of reducing lignin and hemicellulose content, also at low cost, but it may produce high pollution and has high chemical recovery requirements. The organosolv pretreatment process can separate and recover high quality lignin (Sun et al., 2016).

In addition to the issues listed, pretreatment strategies need to be developed, because a single pretreatment type can only change the structure of the material with partial success, meaning the material is still partially inaccessible to enzymatic hydrolysis (Brodeur et al., 2016). Thus, two-stage pretreatment processes are considered to improve the digestibility of enzymatic hydrolysis and maximize the utilization of isolated hemicelluloses and lignin (Sun et al., 2016). Considering the information provided by previous studies, this research will use a two-stage pretreatment process, namely acid pretreatment followed by organosolv pretreatment. According to studies conducted by Brodeur et al. (2016), Mesa et al. (2016), and Kumar and Sharma (2017), the combination of acid and organosolv pretreatment successfully increases the efficiency of the delignification process. In a study by Sheng et al. (2011), 80.99 % of the lignin from empty palm fruit bunches was successfully removed after acid pretreatment (with 1.63 % sulfuric acid concentration), with subsequent organosolv pretreatment (using 65 % ethanol) at an operating temperature of 160 °C for 78 minutes. In a study by Mesa et al. (2011), lignin from sugar cane was successfully removed (0.85 % w/w) after pretreatment with sulfuric acid (at 190 °C), and increased by a

further 17.10% after organosolv pretreatment (using ethanol) at an operating temperature of 175 °C. Therefore, the use of two pretreatment stages can separate hemicellulose and lignin in two-step fractionation and produce cellulose-rich biomass (Mesa et al., 2011). Timung et al. (2016) report that the use of high-temperature pretreatment also results in a decrease in the crystallinity index (CrI) value of cellulose.

In the following stage of this study, statistical and mathematical analysis was conducted to identify the optimum conditions in the second stage of pretreatment by using response surface methodology (RSM). In the study by Sheng et al. (2011), optimization was performed on independent variables such as acid concentration, reaction temperature and process time. Likewise, in the study by Humaidah et al. (2017), optimization with RSM was conducted to determine the optimum conditions for ethanol fermentation from *Borassus flabellifer*, with variations in temperature and pH. Lini et al. (2018) performed optimization of the enzymatic hydrolysis process with variations in pH, temperature and hydrolysis time.

To obtain reducing sugar from SWSPT, cellulose and hemicellulose need to be hydrolyzed into glucose and xylose. In the hydrolysis process, cellulase enzymes are used to convert cellulose into cellobiose via bounded fractions such as endoglucanase and exoglucanase. The cellobiase enzyme is used to convert cellobiose (as the intermediate fraction) into glucose by an unbounded fraction such as  $\beta$ -glucosidase, while the xylanase fraction is converted into xylose by xylanase (Fenila and Yogendra, 2016). In addition, in this study a surfactant was used to optimize enzyme hydrolysis. The addition of a surfactant increases the efficiency of the enzymatic hydrolysis process by reducing the surface tension between the two liquid phases (Chang et al., 2016). In the study by Li et al. (2016), it was shown that the surfactant Tween 80 might improve the cellulase adsorption of corn stover due to the adsorption of Tween 80 to the lignin, which occupies part of the hydrophobic lignin surface in the material. In addition, Tween 80 had a very strong influence on xylanase adsorption and desorption on/from lignin compared to polyethylene glycol.

Given the above factors, this research chose to conduct the pretreatment in two stages. The first stage was performed using an acid solvent (0.2 M dilute sulfuric acid) at 120 °C for 40 minutes; this was then



continued in the second stage with an organic solvent (ethanol) and an alkali (3 % w/w sodium hydroxide) as the catalyst. The use of organic solvents such as ethanol is safer than the alternatives because they have lower toxicity than methanol (Mesa et al., 2011). In addition, the use of sodium hydroxide as a catalyst during the pretreatment of organosolv ethanol enhances the selectivity of ethanol to lignin, as well as the ability of delignification by ethanol. In this study, the effects of different operating conditions on factors such as organic solvent (ethanol) concentration, pretreatment time, and temperature needed to achieve optimum conditions during the pretreatment process (using the response surface method of experiment design), were also investigated. The pretreated solids were then hydrolyzed with varying enzyme formulations. The use of mixtures of cellulase-cellobiase and cellulase-xylanase enzymes with and without the addition of Tween 80 surfactant for each enzyme mixture was expected to increase the performance of the enzymatic hydrolysis.

## Material and Methods

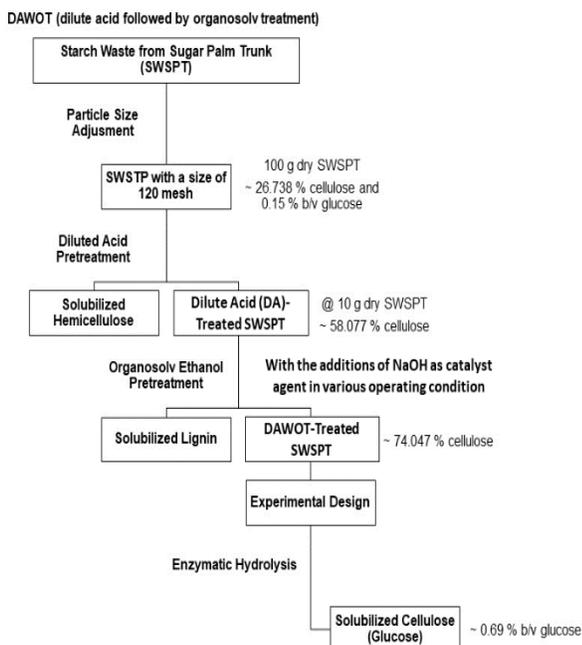


Figure 1. Schematic diagram of the DAWOT process

### Raw material

SWSPT was obtained in the form of dry fiber from Tasikmalaya, West Java, Indonesia. The size of the waste was reduced to 120 mesh and pretreated using

dilute acid followed by organosolv as shown in Figure 1.

### Dilute-acid pretreatment

In the first stage of the acid pretreatment with sulfuric acid, 100 grams of dried solid material and 0.2 M diluted sulfuric acid with a solid-liquid ratio of 1:5 w/w was autoclaved at 120 °C for 40 minutes and then transferred to a 250 ml Erlenmeyer flask. The solid residue obtained was filtered and washed with water until it reached a neutral pH and then dried at a temperature of 40 °C to remove around 5 % moisture.

### Organosolv ethanol pretreatment

In the second stage of the organic solvent pretreatment, 10 gram samples of dried acid-pretreated material were subjected to the organosolv process with material-ethanol and 4N NaOH 3 % (w/w dried fiber) with a solid-liquid ratio of 1:7 w/w. Reaction was then conducted in an autoclave in operating conditions of 30–50 % ethanol concentration, time of 20–60 minutes, and pretreatment temperature of 100–120 °C. The solid residue was then separated using filtration, washed with water until it reached neutral pH, and then dried at a temperature of 40 °C (Mesa et al., 2011). In addition, the lignocellulose content was measured.

### Experimental design

Optimization using RSM was conducted using Minitab 16 statistical software under the licence of ITS Surabaya. A 2<sup>3</sup> full factorial design, central composite design method was conducted using three independent variables: pretreatment duration, temperature, and ethanol concentration. Lignin removal (g/g) was measured as the response to determine the minimum operation conditions of the second stage of pretreatment (see Table 1).

Table 1. Factorial design of the independent variables in the organosolv pretreatment

Independent variable	Symbol	Range and level		
		-1	0	+1
Ethanol concentration (% v/v)	A	30	40	50
Pretreatment time (minutes)	B	20	40	60
Temperature (°C)	C	100	110	120



### Enzymatic hydrolysis

Enzymatic hydrolysis was conducted in a 250 mL Erlenmeyer flask containing 1 g of pretreated material and a 70 mL mixture of cellulase enzymes from *Aspergillus niger* and pure xylanase from *Trichoderma longibrachiatum*, with enzyme amounts of 0.533 U/g and 0.625 U/g respectively. The mixture of cellulase enzymes from *Trichoderma reesei* and cellobiase from *Aspergillus niger* contained 0.893 U/g and 250 U/g of enzymes respectively. Subsequently, a citrate buffer (pH = 5.5) was added. Tween 80 surfactant was added at a ratio of 3:1 to the sample (g/g). The sample was incubated in an incubator shaker at a speed of 125 rpm for 48 hours at 60 °C.

For the SWSPT hydrolysis process, a glucose standard curve was prepared to examine the glucose concentration. Reducing sugar DNS analysis data was gathered from the SWSPT hydrolysis by a mixture of enzymes with and without the addition of Tween 80.

### Analytical procedures

Lignocellulose content was analyzed using gravimetric method refers to Datta (1981). The crystallinity index (CrI) analysis was conducted via X-ray diffraction (XRD) which was equipped with a transmission-type goniometer using CuK $\alpha$  radiation at 40 kV and 30 mA and recorded in the 2 $\theta$  angle in range of 0–50 °. CrI value was calculated using Equation (1) as described by Park et al. (2010):

$$\text{CrI} = (I_{002} - I_{\text{am}}) / I_{002} \times 100\% \quad (1)$$

Analysis of a total reducing sugar concentration was conducted using the 3,5-dinitrosalicylic acid (DNS) method as described by Miller (1959) and the sugar content was investigated using a high-performance liquid chromatograph (HPLC, Agilent Technologies Inc. 1100 Series) equipped with a refractive index detector and Zorbax column (4.6 × 150 mm) which was operated at 30 °C with an acetonitrile/water (75:25 (v/v)) as a mobile phase.

## Results and Discussion

### The effect of dilute-acid pretreatment on SWSPT

The dried fiber SWSPT was first ground with a grinding machine, then mashed with a blender and sieved to obtain a particle size of 120 mesh. The process of sieving raw materials up to 120 mesh size aims to expand the surface of the particles of mass

unity so as to enable easier contact between the enzymes and raw materials, and to increase the cellulose-bond dissociation by the enzymes. This method is supported by research conducted by Caminal et al. (2014), who found that a 120 mesh wheat straw size can increase the speed of enzymatic hydrolysis by 4.7 times compared to un-milled wheat straw, while wheat straw smaller than 160 mesh did not show a significant increase in enzymatic hydrolysis.

**Table 2. Comparison of chemical composition of SWSPT with and without pretreatment**

Component	Content (%)		
	Without pre-treatment	After acid pre-treatment	After organosolv pre-treatment
Hot water soluble	26.686	19.986	16.028
Cellulose	26.738	58.077	74.047
Hemicellulose	20.387	3.528	2.312
Lignin	24.739	17.617	7.142
Ash	1.451	0.791	0.471

Following sieving, acid pretreatment with 0.2 M H<sub>2</sub>SO<sub>4</sub> concentration was performed in an autoclave for 40 min at 120 °C. The use of acid solvents aims to improve the efficiency of the organosolv process by removing the hemicellulosic fraction in the first stage of pretreatment. In addition, hemicellulosic removal is also economically and technologically advantageous, because it can reduce the use of chemicals in the next stage (organosolv pretreatment). In the acid pretreatment, the use of a lower temperature (120 °C) than in the previous study of Mesa et al. (2011) was because of the limited operating temperature in the autoclave used. In addition, the use of lower temperatures can reduce energy use. In this study, there was a decrease in the SWSPT hemicellulose levels of 88.66 % (w/w), similar to the results of the study conducted by Mesa et al. (2011), in which hemicellulose clearance in bagasse waste was 87.5 % (w/w). According to a study conducted by Avci et al. (2013), xylose concentration will decrease with increasing acid concentration (from 0.75 % H<sub>2</sub>SO<sub>4</sub> to 1 % H<sub>2</sub>SO<sub>4</sub>) and rising temperature (from 140 ° to 200 °C). However, in this study, an acid concentration of 0.2 M (equivalent to 0.93 %) was used because cellulose has a crystalline structure that is more acid-resistant than hemicellulose, while hemicellulose has a random amorphous structure with a complex



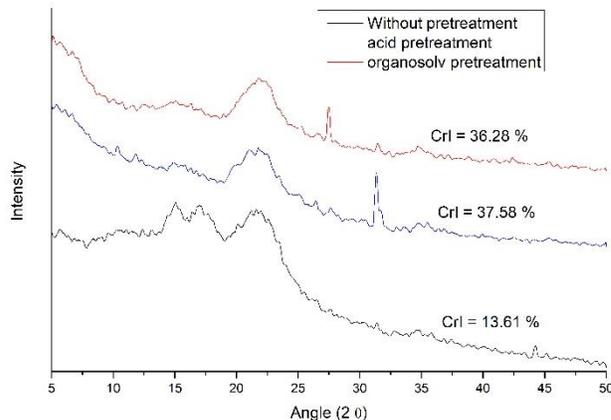
chemical composition and is easily hydrolyzed into sugar.

**Effect of organosolv pretreatment using NaOH as the catalyst agent**

Ethanol was used as an organic solvent in the second stage of the pretreatment process, because it is a renewable solvent capable of producing higher recovery solids, protects the cellulosic fraction, and increases the porosity of residual solids and recovery of high-quality lignin fractions with potential for use in certain industrial applications (Wildschut et al., 2013). The addition of 3 % (dry fiber) NaOH solution increases glucose yield after enzymatic hydrolysis, in accordance with a study conducted by Zhao et al. (2009), which showed an elevated glucose yield of 30.4 % after enzymatic hydrolysis at 3 % NaOH w/w. Table 2 shows that the lignin level in SWSPT can be reduced by 71.13 % (w/w) after organosolv pretreatment. This removal of lignin shows an increase compared with the acid pretreatment, which only achieved a removal of 28.79 % (w/w). In another study (Mesa et al., 2011), the percentage of lignin removal with an organosolv pretreatment temperature of 185 °C in sugar cane dregs was 17.1 % (w/w), while in a study by Sheng et al. (2011), treatment of empty palm fruit bunches using 1.63% sulfuric acid and organic solvent ethanol 65 % at 160 °C for 78 minutes was able to eliminate 80.99 % of lignin (w/w). Table 2 also shows an increase in cellulose content of 63.891 %, which led to the higher concentration of reducing sugar obtained.

**Effect of two-stage pretreatment on crystallinity index cellulose (CrI)**

Cellulose is a complex biopolymer consisting of amorphous and crystalline cellulose. It is known that enzymes digest amorphous cellulose first and then digest the more difficult crystalline cellulose more slowly (Park et al., 2010). According to Barlianti et al. (2015), CrI is one of the important parameters that describes the crystalline content of cellulose. Crystalline cellulose has limited accessibility to water and chemical solutions. Chemical destruction occurs in the amorphous phase and on the crystalline surface. Cellulose and amorphous cellulose crystalline compositions need to be known in order to formulate enzyme mixtures for the cellulose hydrolysis process.



**Figure 2. X-Ray diffraction analysis of SWSPT before and after pretreatment**

From Figure 2 it can be seen that the results of the analysis of both wastes, both before and after pretreatment, show that  $I_{002}$  is the crystalline fraction of cellulose at the maximum diffraction peak intensity of  $2\theta = 22.5^\circ$  (the most common cellulose form in nature) (Karapatsia et al., 2017) and  $I_{am}$  is the amorphous fraction of cellulose at the intensity of the minimum diffraction peak of  $2\theta = 18.7^\circ$  (Yoshida et al., 2014). The peak seen at  $2\theta = 27.45^\circ$  in Figure 1 is presumed to be the peak of hemicellulose (Hamada et al., 2013). This is in accordance with the analysis results, which indicate that hemicellulose levels in SWSPT fell from 20.39 % to 2.31 %. The crystallinity index values were calculated using Equation (1), as shown in Table 3:

**Table 3. CrI of SWSPT with different variables**

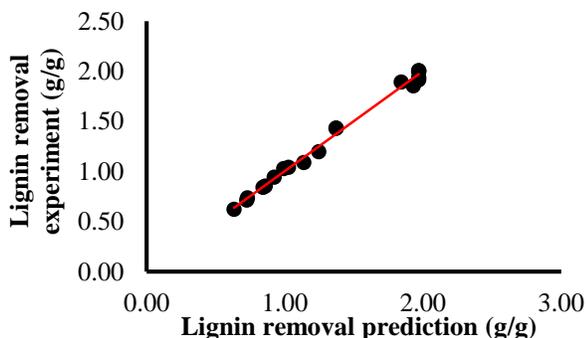
Variable	$I_{002}$	$I_{am}$	CrI (%)
Without pretreatment	357.38	308.74	13.61
After acid pretreatment	239.10	149.24	37.58
After organosolv pretreatment	227.04	144.68	36.28

After acid pretreatment, the SWSPT CrI values increased from 13.61 % to 37.58 %, as a result of the removal of extractive water and remaining hemicellulose from the crystalline cellulose during the acid pretreatment process (Timung et al., 2016). The decrease in the cellulose crystallinity index from acid pretreatment to organosolv pretreatment (from 37.58 % to 36.28 %) was due to the use of high-temperature pretreatment, i.e. 107°C, as proven by a higher CrI value (42.25 %.) at a pretreatment temperature of 92.68 °C.



**Response surface methodology**

In order to establish the significance of the independent variables, a P-value lower than 0.05 is sought. From Table 4, it can be seen that the significant independent variables are ethanol concentration, time and temperature. It can be concluded that high concentrations of ethanol, longer times and higher temperatures can decrease the lignin content in the second stage pretreatment process using ethanol. However, one interaction, that between time and pretreatment temperature, is insignificant. In Figure 4 (C), the response surface of the effect of temperature and pretreatment time on the decrease of lignin can be seen. In addition, Figures 4 (A) and (B) show that there are optimum operating conditions with regard to the pretreatment time and temperature variables for EtOH concentration, but the equation produced by the response shows that the optimum pretreatment time and temperature points are outside the range of levels in this study. Therefore, in this study, a differentiation of Equation 2 was employed, meaning optimum operating conditions were obtained at an ethanol concentration of 30.35 % v/v, pretreatment time of 33 minutes and temperature of 107 °C. This compares to a study conducted by Mesa et al. (2016), in which optimum conditions for organosolv pretreatment of bagasse were achieved using a 30% ethanol concentration for 60 min and at an operating temperature (using a semi-pilot reactor) of 195 °C.



**Figure 3. Parity chart of lignin removal. Experimental data versus the predicted value.**

Another study by Sheng et al. (2011) found that the optimum conditions for organosolv pretreatment of empty palm fruit bunch material were 65 % ethanol organic solvent and 1.63 % sulfuric acid as a catalyst, at 160 °C for 78 min. In contrast to the findings of these studies, the conditions in this study can save

energy and reduce equipment costs, given that this research process only uses an autoclave with a maximum temperature safety limit of 127 °C.

The accuracy of the experimental results in this model can be seen from the R-square value; the more accurate an experiment, the closer this value will be to 100 %. In this study, a fairly high R-square value of 99.8 % was obtained (see Figure 3). High R-square values also show that the model obtained will be able to provide good response estimations within the range used in the study (Sheng et al., 2011). In addition, a small error (0.02964) was obtained for the organosolv pretreatment.

**Table 4. Analysis of Variance (ANOVA) results from the CCD experiment**

Source	Degree of Freedom	Sum of Squares	Mean Square	F-Value	P-value
PModel	9	4.75781	0.52865	160.54	0.000 s
Linear	3	1.56071	0.52024	157.98	0.000 s
A, EtOH (% v/v)	1	0.95420	0.95420	289.77	0.000 s
B, t (menit)	1	0.31142	0.31142	94.57	0.000 s
C, T (°C)	1	0.29509	0.29509	89.61	0.000 s
Square	3	3.08782	1.02927	312.56	0.000 s
A <sup>2</sup>	1	0.39905	0.39905	121.18	0.000 s
B <sup>2</sup>	1	1.60321	1.60321	486.85	0.000 s
C <sup>2</sup>	1	1.94542	1.94542	590.77	0.000 s
2-Way Interaction	3	0.10928	0.03643	11.06	0.002 s
AB	1	0.05237	0.05237	15.90	0.003 s
AC	1	0.05667	0.05667	17.21	0.002 s
BC	1	0.00024	0.00024	0.07	0.794 ns
Error	9	0.02964	0.00329		
Lack-of-Fit	5	0.02199	0.00440	2.30	0.220
Pure Error	4	0.00765	0.00191		
Total	18	4.78745			

s = significant (P < 0.05); ns = not significant (P > 0.05)

From the calculations using Minitab software, Equation (2) was used to calculate the optimum conditions of the SWSPT organosolv pretreatment process.

$$\text{Lignin removal (g/g)} = -38,30 - 0,0039 A + 0,039 B + 0,7462 C - 0,001638 A^2 - 0,000821 B^2 - 0,003616 C^2 + 0,000405 AB + 0,000842 AC + 0,000027 BC \quad (2)$$



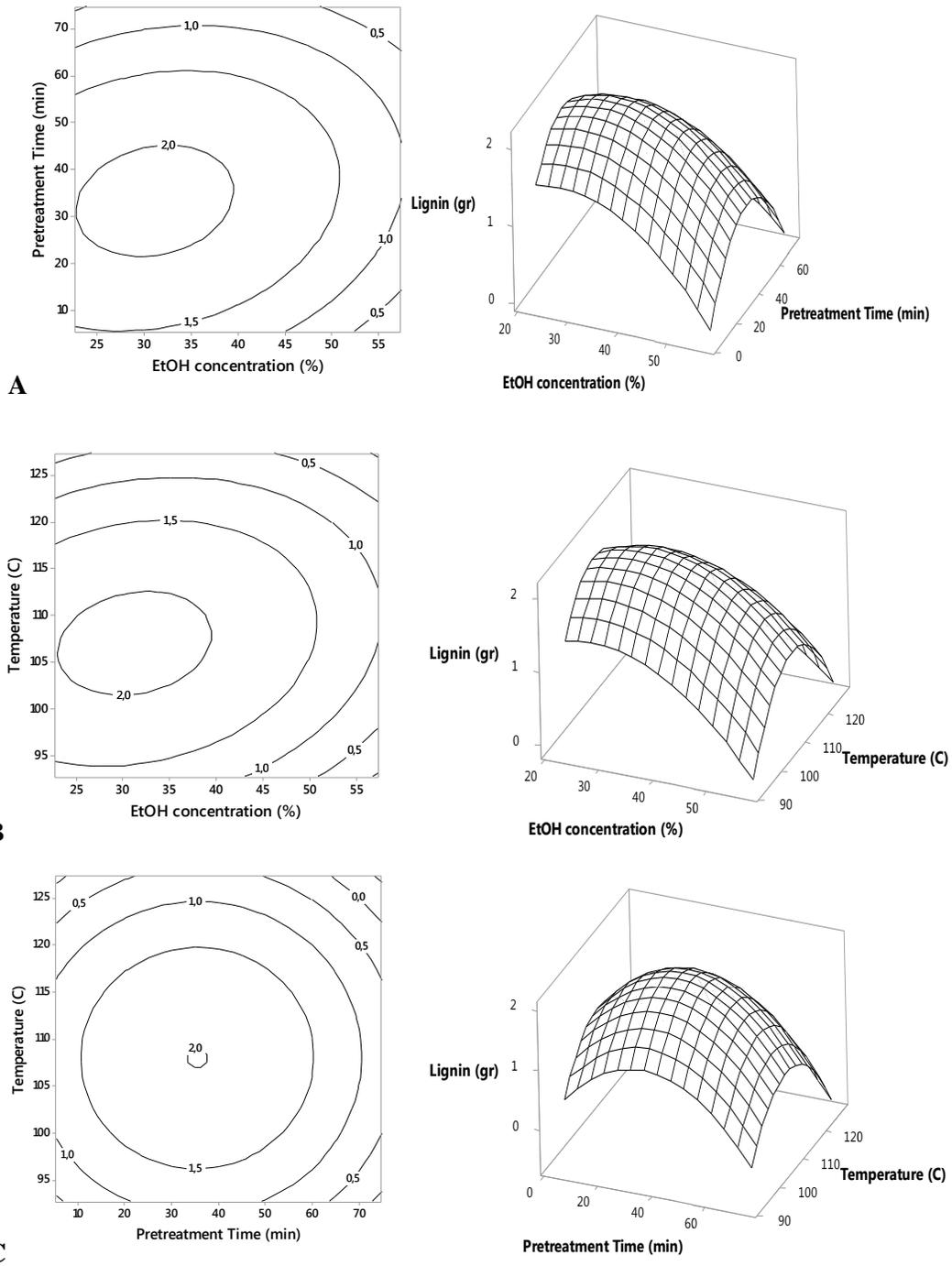
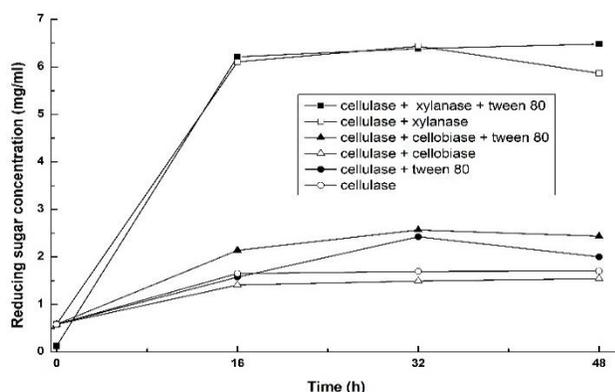


Figure 4. Graphs of surface response and contours

## Enzymatic hydrolysis

**Table 5. Reducing sugar from the hydrolysis of SWSPT by a mixture of enzymes with and without the addition of Tween 80**

Variant	Reducing sugar concentration (mg/mL)
Cellulase + Xylanase + Tween 80	6.480
Cellulase + Xylanase	5.782
Cellulase + Cellobiase + Tween 80	2.111
Cellulase + Cellobiase	1.155
Cellulase + Tween 80	1.647



**Figure 5. Effect of hydrolysis time on reducing sugar concentration**

Reducing sugar concentration resulted in enzymatic hydrolysis at 48 h are presented in Table 5. Figure 5 shows that the glucose concentration tends to increase during hydrolysis. In this process, the mixture of cellulase and xylanase enzymes with the addition of Tween 80 produces the highest reducing-sugar concentration value of 6.480 mg/mL, while the cellulase and cellobiase mixture with Tween 80 produces 2.111 mg/mL. The higher reducing-sugar concentration was obtained because in addition to glucose as the main product produced in this enzymatic hydrolysis, a small amount of xylose was also formed. Moreover, there was no inhibition by  $\beta$ -glucosidase in the enzymatic hydrolysis. Alencar et al. (2017) report that inhibition by  $\beta$ -glucosidase did not occur in their study because the yield of cellulose obtained was approximately 90 % after 48 hours when pretreatment was carried out with acid solvents and surfactants. Meanwhile, the failure of the addition of cellobiase to increase hydrolysis was because the concentration of cellobiose retained at low

concentrations during the hydrolysis process experienced a rapid increase in activity. Therefore, inhibition of feedback caused by cellulosic accumulation was greatly reduced, resulting in a decrease in sugar concentration because the cellobiase enzyme did not perform well. In addition, according to Barlianti et al. (2015), the conversion of crystalline cellulose into glucose requires exoglucanase enzymes, whereas the enzyme required to convert amorphous cellulose into glucose is endoglucanase. This is in line with the results of the XRD analysis in this study, which show that most of the cellulose contained in the two raw materials after organosolv pretreatment is amorphous cellulose. Therefore, endoglucanase is more necessary for breaking the long chain of amorphous cellulose into shorter chains than exoglucanase, which hydrolyzes the 1,4-glycosidyl bond into a cellobiose form.

The increase in hydrolysis yield to 10.77 % at 48 hours was also caused by the addition of the surfactant Tween 80, compared to when it was not used. This result is in line with the study conducted by Alencar et al. (2017), which found that the use of Tween 80 may increase the concentration of reducing sugar by 50 %.

The reasons for the addition of surfactants to enzymatic hydrolysis processes include the fact that surfactants can increase stability and prevent the denaturation of enzymes; they may affect substrate structures and make substrates more accessible during hydrolysis by reducing surface tension; and they may affect substrate-enzyme relationships, which will help increase the effectiveness of cellulose conversion into reducing sugars. The surfactant mass selection is based on research (Zhou et al., 2015) which states that non-ionic surfactants added to the hydrolysis process will produce significant results, but that the addition of over 5 grams will not have a significant effect due to the saturated solubility of the surfactant.

An increase in reducing-sugar concentration also occurs in the use of enzyme mixtures using cellobiase enzymes, compared to the absence of these enzyme mixtures. In this study, an increase of 22.0 % was obtained, while utilization of cellobiase increased by 18.9 %. However, the increase is lower than with the use of a mixture of xylanase enzymes by up to 74.6 %, while utilization of xylanase increases to 35 %. From Table 6, it can be concluded that the glucose content obtained from the cellulose conversion results in greater levels (0.69 %) than the xylose levels obtained from the conversion of the hemicellulose substrate.



**Table 6. HPLC test results on SWSPT hydrolysate**

Sample	Parameter	Result (% w/v)
Un-pretreated	Glucose	0.15 ± 0.53
	Xylose	-
After organosolv pretreatment	Glucose	0.69
	Xylose	-

The results support those of gravimetric analysis (the Chesson-Datta method), in which the hemicellulose produced after pretreatment decreases by 2.312 % and the levels are much lower than those of cellulose. Meanwhile, the hydrolysate of un-pretreated SWSPT does not produce glucose or xylose. This is because the cellulose and hemicellulose are still bound with lignin and so cannot be converted into glucose and xylose.

## Conclusion

The combination of acid and organosolv pretreatment with optimum conditions of 33 min, 107 °C, and of 30.35 % (v/v) ethanol was an efficient technique to hydrolyze SWSPT into enzymatic reducing sugar. The decrease in the crystallinity index from 37.58 % to 36.28 % caused the amorphous content of cellulose to increase, which led to easier access of the enzymes to the amorphous cellulose of the substrate. This could then be converted to reducing sugar, with the highest yield of 0.016 g reducing sugar/g of substrate achieved by using a mixture of cellulase and xylanase enzymes with the addition of Tween 80, resulting in a removal of lignin of 80.99 % (w/w) and a removal of hemicellulose of 87.38 % (w/w).

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## Contribution of Authors

Widjaja T: Conceived Idea, Designed Research Methodology, Manuscript final reading and approval  
Altway A: Data Interpretation, Statistical Analysis, Manuscript final reading and approval

Pudjiastuti L: Literature Search, Literature Review  
Lini FZ: Data Collection, Data Interpretation, Statistical Analysis, Manuscript Writing  
Nury DF.: Literature Search, Literature Review  
Iswanto T: Provide SWSPT, Designed Research Methodology, Statistical Analysis, Manuscript Writing.

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**Conflict of Interest:** None.

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## References

- Alencar BRA, Rocha JMTS, Rocha GJM and Gouveia ER, 2017. Effect of tween-80 addition in dilute acid pretreatment of waste office paper on enzymatic hydrolysis for bioethanol production by SHF and SSF processes. *Cellul. Chem. Technol.* 51: 121–126.
- Avci A, Saha BC, Kennedy GJ and Cotta MA, 2013. Dilute sulfuric acid pretreatment of corn stover for enzymatic hydrolysis and efficient ethanol production by recombinant *Escherichia coli* FBR5 without detoxification. *Bioresour. Technol.* 142: 312–319.
- Barlianti V, Dahnum D, Hendarsyah H and Abimanyu H, 2015. Effect of alkaline pretreatment on properties of lignocellulosic oil palm waste. *Procedia Chem.* 16: 195–201.
- BPS Tasikmalaya, 2015. Kabupaten Tasikmalaya Dalam Angka 2015. Kabupaten Tasikmalaya, Indonesia.
- Brodeur G, Telotte J, Stickel JJ and Ramakrishnan S, 2016. Two-stage dilute-acid and organic-solvent lignocellulosic pretreatment for enhanced bioprocessing. *Bioresour. Technol.* 220: 621–628.
- Caminal G, Hui L, Xiong H, Du K, Tacey SA, Demers A, Doane R, Turon X and Rojas OJ, 2014. Alkali pretreated of wheat straw and its enzymatic hydrolysis. *Bioresour. Technol.* XXVII, 73–122.
- Chang K, Chen X, Han Y, Wang X, Potprommanee L, Ning X, Liu J, Sun J, Peng Y, Sun S and Lin Y, 2016. Synergistic effects of surfactant-assisted ionic liquid pretreatment rice straw. *Bioresour. Technol.* 214: 371–375.



- Datta R, 1981. Acidogenic fermentation of lignocellulose-acid yield and conversion of components. *Biotechnol. Bioeng.* 23: 2167–2170.
- Fenila F and Yogendra S, 2016. Optimal control of enzymatic hydrolysis of lignocellulosic biomass. *Resour. Technol.* 2, S96–S104.
- Hamada Y, Yoshida K, Asai R, Hayase S, Nokami T, Izumi S and Itoh T, 2013. A possible means of realizing a sacrifice-free three component separation of lignocellulose from wood biomass using an amino acid ionic liquid. *Green Chem.* 15: 1863.
- Humaidah N, Widjaja T, Budisetyowati N and Amirah H, 2017. Comparative study of microorganism effect on the optimization of ethanol production from palmyra sap (*Borassus flabellifer*) using response surface methodology. *Chem. Eng. Transactions.* 56: 1789-1794
- Jönsson LJ and Martín C, 2016. Pretreatment of lignocellulose: formation of inhibitory by-products and strategies for minimizing their effects. *Bioresour. Technol.* 199: 103–112.
- Karapatsia A, Pappas I, Penloglou G, Kotrotsiou O and Kiparissides C, 2017. Optimization of dilute acid pretreatment and enzymatic hydrolysis of *Phalaris aquatica* L. lignocellulosic biomass in batch and fed-batch processes. *Bioenergy Res.* 10: 225–236.
- Kumar AK and Sharma S, 2017. Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. *Bioresour. Bioprocess.* 4(7): 1-19.
- Li Y, Sun Z, Ge X and Zhang J, 2016. Effects of lignin and surfactant on adsorption and hydrolysis of cellulases on cellulose. *Biotechnol. Biofuels.* 9(20): 1-9.
- Liang J, Chen X, Wang L, Wei X, Wang H, Lu S and Li Y, 2017. Subcritical carbon dioxide-water hydrolysis of sugarcane bagasse pith for reducing sugars production. *Bioresour. Technol.* 228: 147–155.
- Lini FZ, Widjaja T, Hendriani N, Altway A, Nurkhamidah S and Tansil Y, 2018. The effect of organosolv pretreatment on optimization of hydrolysis process to produce the reducing sugar. *MATEC Web Conf.* 154.
- Mesa L, González E, Cara C, González M, Castro E and Mussatto SI, 2011. The effect of organosolv pretreatment variables on enzymatic hydrolysis of sugarcane bagasse. *Chem. Eng. J.* 168: 1157–1162.
- Mesa L, López N, Cara C, Castro E, González E and Mussatto SI, 2016. Techno-economic evaluation of strategies based on two steps organosolv pretreatment and enzymatic hydrolysis of sugarcane bagasse for ethanol production. *Renew. Energy.* 86: 270–279.
- Miller GL, 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31: 426–428
- Park S, Baker JO, Himmel ME, Parilla PA and Johnson DK, 2010. Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. *Biotechnol. Biofuels.* 3: 1–10.
- Sheng C, Teng H, Teong K, Brosse N, Universite N and Aiguillettes B, 2011. Evaluation and optimization of organosolv pretreatment using combined severity factors and response surface methodology. *Biomass Bioenergy.* 35: 4025–4033.
- Sun S, Sun S, Cao X and Sun R, 2016. The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. *Bioresour. Technol.* 199: 49–58.
- Timung R, Deshavath NN, Goud VV and Dasu VV, 2016. Effect of subsequent dilute acid and enzymatic hydrolysis on reducing sugar production from sugarcane bagasse and spent citronella biomass. *J. Energy.* 2016: 1-12.
- Wildschut J, Smit AT, Reith JH and Huijgen WJJ, 2013. Ethanol-based organosolv fractionation of wheat straw for the production of lignin and enzymatically digestible cellulose. *Bioresour. Technol.* 135: 58–66.
- Yoshida M, Liu Y, Uchida S, Kawarada K, Ichinose H, Kaneko S and Fukuda K, 2008. Effects of cellulose crystallinity, hemicellulose, and lignin on the enzymatic hydrolysis of *Miscanthus sinensis* to monosaccharides. *Biosci. Biotechnol. Biochem.* 72(3): 805–810.
- Zhao X, Cheng K and Liu D, 2009. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Appl. Microbiol. Biotechnol.* 82: 815–827.
- Zhou Y, Chen H, Qi F, Zhao X and Liu D, 2015. Non-ionic surfactants do not consistently improve the enzymatic hydrolysis of pure cellulose. *Bioresour. Technol.* 182: 136–143.

