

Optimization of The Organosolv Pretreatment of Yellow Poplar for Bioethanol Production by Response Surface Methodology¹

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ABSTRACT

We investigated the optimization of the organosolv pretreatment of yellow poplar for bioethanol production. Response surface methodology was used to determine the optimal conditions of three independent variables (reaction temperature, reaction time, and sulfuric acid (SA) concentration). Reaction temperature is the most significant variable in the degradation of xylan and lignin in the presence of an acid catalyst, and ethanol production increased with a decrease in the lignin content. The highest ethanol concentration (42.80 g/ℓ) and theoretical ethanol yield (98.76%) were obtained at 152°C (2.5 bar) with 1.6% SA for 16 min. However, because of excessive degradation of the raw material, the overall ethanol yield was less than under other pretreatment conditions which has approximately 50% of WIS recovery rate after pretreatment. The optimal conditions for the maximum overall ethanol yield (146°C with 1.22% SA for 15.9 min) were determined with a predicted yield of 17.11%, and the experimental values were very close (17.15%). Therefore, the quadratic model is reliable.

Keywords : organosolv pretreatment, yellow poplar, overall ethanol yield, simultaneous saccharification and fermentation, response surface methodology

1. INTRODUCTION

Renewable alternatives to petroleum-based fuel are of increasing interest in research and development. Bioethanol produced from corn grain is the most widely used renewable trans-

portation fuel, although there has been considerable debate regarding the effect of bioethanol production on the food supply (Hill *et al.*, 2006). The annual worldwide production of lignocellulosic biomass has been estimated to be 1×10^{10} MT (Sanchez and Cardona, 2008).

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Conversion of lignocellulosic biomass to ethanol is a reliable alternative to corn-based ethanol with diminishing net energy balance and dramatic decreases in GHG emissions (Farrell *et al.*, 2006; Halvorsen *et al.*, 2009). However, pretreatment is required in the bioconversion processes to increase yields and reduce costs (Saddler *et al.*, 1993).

Thus, various pretreatment processes have been developed to enhance lignocellulosic bioethanol conversion (Cara *et al.*, 2008; Wyman *et al.*, 2005; Alvira *et al.*, 2010). Organosolv pretreatment is a promising method to enhance the recovery of glucose by improving the digestibility of high-lignin lignocellulosic biomass (Chum *et al.*, 1990; Koo *et al.*, 2011; Park *et al.*, 2010). Organosolv pretreatment makes cellulose more accessible to enzymes by extracting lignin from biomass (Holtzapfel and Humphrey, 1984). In addition, organosolv lignin extracted by the organosolv pretreatment process has high purity and valuable applications (Koo *et al.*, 2011; Domínguez *et al.*, 2008; El Hage *et al.*, 2010). Organosolv pretreatment is commonly performed with low boiling point organic solvent (ethanol, methanol) and a catalyst that is an inorganic acid or base (hydrochloric acid, sulfuric acid, sodium hydroxide) (Koo *et al.*, 2011; Zhao *et al.*, 2009). Several organosolv pretreatment studies have examined various lignocellulosic biomasses, such as poplar, *Miscanthus* and wheat straw (Koo *et al.*, 2011; Park *et al.*, 2010; El Hage *et al.*, 2010; Sidiras and Koukios, 2004). However, previous organosolv pretreatment studies are limited by

insufficient optimization of conditions.

The optimization of pretreatment conditions is one of the most important stages for experimental scale-up and industrial applications (Kim *et al.*, 2010; Perez *et al.*, 2008). Response surface methodology (RSM) is an effective optimization tool that maps response surfaces over regions of interest. Among RSMs, central composite design (CCD) is well suited for fitting a quadratic surface and typically works well for process optimization. A CCD with three factors requires 17 runs with a center point of 3 replicates. For these reasons, RSM and CCD have been successfully applied to biomass pretreatment (Kim *et al.*, 2010; Goh *et al.*, 2011; Maache-Rezzoug *et al.*, 2011; Kim *et al.*, 2013).

The main purpose of this experiment was to determine the optimal conditions to produce maximum lignocellulosic bioethanol by organosolv pretreatment and simultaneous saccharification and fermentation from yellow poplar. For this purpose, RSM based on three variables (reaction temperature, reaction time, acid concentration) and CCD were adopted to determine optimal pretreatment conditions by measuring the water-insoluble solid recovery rate and ethanol concentration. Fundamental analyses of the solid fraction were performed after pretreatment to support condition optimization.

2. MATERIALS and METHODS

2.1. Raw materials

Twenty year-old yellow poplar (*Liriodendron*

Table 1. Central composite design varying on three factors: Pretreatment temperature, reaction time and sulfuric acid (SA) concentration

| Std | Variables | | | Code levels | | |
|-----|------------------|---------------------|------------------------|----------------|----------------|--------------------|
| | Temperature (°C) | Reaction time (min) | Acid concentration (%) | Temperature | Reaction time | Acid concentration |
| | X ₁ | X ₂ | X ₃ | X ₁ | X ₂ | X ₃ |
| 1 | 128 | 4 | 0.4 | - 1 | - 1 | - 1 |
| 2 | 152 | 4 | 0.4 | 1 | - 1 | - 1 |
| 3 | 128 | 16 | 0.4 | - 1 | 1 | - 1 |
| 4 | 152 | 16 | 0.4 | 1 | 1 | - 1 |
| 5 | 128 | 4 | 1.6 | - 1 | - 1 | 1 |
| 6 | 152 | 4 | 1.6 | 1 | - 1 | 1 |
| 7 | 128 | 16 | 1.6 | - 1 | 1 | 1 |
| 8 | 152 | 16 | 1.6 | 1 | 1 | 1 |
| 9 | 120 | 10 | 1 | - 1.68 | 0 | 0 |
| 10 | 160 | 10 | 1 | 1.68 | 0 | 0 |
| 11 | 140 | 0 | 1 | 0 | - 1.68 | 0 |
| 12 | 140 | 20 | 1 | 0 | 1.68 | 0 |
| 13 | 140 | 10 | 0 | 0 | 0 | - 1.68 |
| 14 | 140 | 10 | 2 | 0 | 0 | 1.68 |
| 15 | 140 | 10 | 1 | 0 | 0 | 0 |
| 16 | 140 | 10 | 1 | 0 | 0 | 0 |
| 17 | 140 | 10 | 1 | 0 | 0 | 0 |

tulipifera) stems grown in Hwaseong-si, Gyeong-ki do, South Korea, were provided by the Korea Forest Research Institute. Raw materials were milled and screened to less than 0.5 mm using a Cutting Mill pulverisette 15 (FRITSCH GmbH, Germany) and stored in plastic bags at 4°C (less than 10% the initial moisture content).

2.2. Organosolv pretreatment

Pretreatment was conducted in a 1 ℓ reactor (Bolted Closure Vessels, Hanwoul Engineering Co. Ltd., Korea) made of stainless steel (SUS

316) and containing an inner thermocouple to control and measure the internal temperature. The reactor was loaded with 25 g of wood powder and 500 ml of a 50 : 50% ethanol : water mixture (v/v) containing sulfuric acid at concentrations from 0 to 2% (w/w) as an acid catalyst. The reaction temperature was electrically controlled from 120°C to 160°C by an external controller. The reactor was preheated to the reaction temperature for 25 min to 35 min and the reaction time (0 min to 20 min) and pressure (from 0.5 to 3 bar) were monitored when the desired temperature was achieved. After pretreatment, the reactor was placed in an

Table 2. Composition analysis of pretreated solid fraction and liquid fraction after SSF process

| Std | Composition of solid fraction after pretreatment (%) | | | | | Composition of liquid fraction after SSF process (72h) | | | | | |
|---------|--|---------------------------|-------------------------------|---------------|---------------------|--|-----------------|------------------|------------------------------------|------------------------------------|---------------------|
| | Glucan ^a (%) | Xylan ^a (%) | K. lignin ^a (%) | A.S.L. (%) | WIS recovery (%) | Glucose (g/l) | Xylose (g/l) | Ethanol (g/l) | Y ₁ ^b (%) | Y ₂ ^c (%) | WIS recovery (%) |
| 1 | 44.47 (11.06) | 17.12 (10.90) | 19.86 (34.14) | 3.45 | 79.16 | 0.015 | 0.288 | 6.67 | 29.41 | 5.28 | 83.05 |
| 2 | 71.64 (7.86) | 7.69 (74.27) | 10.93 (76.68) | 1.67 | 50.90 | - | 0.492 | 32.33 | 88.48 | 16.46 | 21.06 |
| 3 | 47.08 (14.40) | 12.54 (40.68) | 18.71 (43.59) | 3.01 | 71.95 | - | 0.512 | 9.69 | 40.35 | 6.97 | 71.36 |
| 4 | 80.42 (11.12) | 5.44 (84.35) | 8.14 (85.09) | 1.24 | 43.74 | - | 0.324 | 37.27 | 90.88 | 16.30 | 13.42 |
| 5 | 55.94 (4.06) | 12.09 (46.04) | 19.10 (45.67) | 2.67 | 67.88 | - | 0.470 | 11.64 | 40.80 | 7.90 | 61.88 |
| 6 | 84.14 (11.24) | 4.03 (88.92) | 7.99 (86.03) | 1.07 | 41.75 | - | 0.245 | 38.85 | 90.54 | 16.22 | 11.37 |
| 7 | 68.64 (4.88) | 8.33 (69.97) | 14.02 (67.79) | 2.00 | 54.85 | - | 0.473 | 27.48 | 78.50 | 15.07 | 34.18 |
| 8 | 84.98 (20.57) | 2.19 (94.66) | 6.87 (89.35) | 0.85 | 37.00 | - | 0.123 | 42.80 | 98.76 | 15.84 | 11.82 |
| 9 | 47.57 (10.16) | 14.36 (29.42) | 23.68 (25.86) | 2.53 | 74.74 | - | 0.426 | 7.77 | 32.02 | 5.81 | 75.46 |
| 10 | 90.40 (10.91) | 1.98 (94.91) | 5.60 (90.86) | 0.87 | 39.01 | - | 0.094 | 38.71 | 83.96 | 15.10 | 10.10 |
| 11 | 62.66 (6.40) | 9.55 (62.86) | 15.54 (61.52) | 3.08 | 59.12 | - | 0.520 | 19.73 | 61.74 | 11.67 | 44.31 |
| 12 | 81.42 (7.65) | 6.31 (81.38) | 8.77 (83.50) | 1.52 | 44.89 | - | 0.340 | 36.63 | 88.21 | 16.44 | 14.27 |
| 13 | 40.91 (-0.36) | 15.21 (2.89) | 26.12 (-6.27) | 2.63 | 97.11 | - | 0.130 | 9.07 | 43.48 | 8.81 | 84.33 |
| 14 | 86.27 (13.82) | 1.75 (95.46) | 10.53 (82.57) | 0.82 | 39.54 | - | 0.081 | 38.37 | 87.21 | 15.17 | 11.92 |
| 15 | 72.39 (7.65) | 6.90 (77.09) | 11.83 (74.97) | 1.67 | 50.49 | - | 0.455 | 30.92 | 83.75 | 15.61 | 19.94 |
| 16 | 73.91 (9.61) | 6.53 (79.21) | 11.40 (76.89) | 1.62 | 48.41 | - | 0.361 | 32.20 | 85.42 | 15.59 | 18.97 |
| 17 | 72.19 (9.25) | 7.23 (76.33) | 10.68 (77.74) | 1.79 | 49.76 | - | 0.463 | 32.18 | 87.41 | 16.01 | 22.46 |
| Control | 39.58 | 15.21 | 23.87 | 2.99 | - | - | 0.136 | 2.36 | 11.69 | 2.24 | 86.12 |

^a Percentage of degraded rate of components after pretreatment was calculated followed by Eq. (1).

^b Theoretical ethanol yield based on the glucose content of pretreated solid fraction was calculated followed by Eq. (2).

^c Overall ethanol yield based on initial input measured by WIS recovery rate after pretreatment and concentration of produced ethanol was calculated followed by Eq. (4).

ice chamber to cool to below room temperature within 20 min. Pretreated mixtures were washed with a double volume of distilled water to remove degraded products and solvents (Holtzapple and Humphrey, 1984), and divided into the solid fraction (pretreated biomass) and liquid fraction (aqueous-organosoluble fraction) by filtration using Advantec No. 2 filter paper (Toyo Roshi Kaisha Ltd., Japan). The water-insoluble solid (WIS) recovery rate (Table 2) was measured based on the filtered WIS weight after pretreatment or the simultaneous saccharification and fermentation (SSF) process. The degraded components (%) from the solid

fraction during pretreatment were calculated by Eq. (1) and are presented in Table. 2.

$$\text{Degraded components (\%)} = 100 - \frac{\text{weight recovery (\%)} \times \text{component of solid residues (g)}}{\text{component of raw material (g)}} \quad (1)$$

2.3. Simultaneous saccharification and fermentation

SSF was performed to evaluate the efficiency of organosolv pretreatment (Lee *et al.*, 2008). Cellulase (NS-50013, enzyme loading of 30

Filter Paper Units (FPU)/g substrate), β -glucosidase (NS-50010, 0.3 times the NS-50013 input amount), and *Saccharomyces cerevisiae* (ATTC[®] 26603, 1 g/l) were used for the SSF procedure. Both NS-50013 and NS-50010 were kindly provided by Novozyme Korea Ltd. A total of 5 g of solid fraction was placed in a 50 ml working volume including enzymes, yeast, and trace elements (0.1 ml/g substrate) and 50 mM acetate buffer in a 250 ml Erlenmeyer flask. Incubation was performed in a shaking incubator at 30°C, 150 rpm for 72 h.

2.4. Statistical approach

Analyses were conducted to determine the optimal conditions of organosolv pretreatment to produce the highest bioethanol yield by the SSF process. Three independent variables, reaction temperature (x_1 , °C), reaction time (x_2 , min) and sulfuric acid (SA) concentration (x_3 , % (w/w)) based on 2^3 full factorial CCD for RSM were performed using Design Expert 8.0.6 software (Stat-Ease, Inc., MN, USA). Theoretical ethanol yield (Y_1 , %) and overall ethanol yield (Y_2 , %) were the dependent variables. Fifteen conditions composed of six axial points are listed in Table 1, and triplication was performed at the central point (140°C, 10 min, 1% (w/w)).

2.5. Analytical methods

Carbohydrate compositions of raw material and solid fractions after pretreatment were

measured in accordance with the National Renewable Energy Laboratory Chemical Analysis and Testing Standard (Sluiter *et al.*, 2004). The amounts of glucose, xylose, and ethanol were analyzed by high performance liquid chromatography (LC 1100, Agilent, USA) at 40°C with 50 mM H₂SO₄ as the eluent at a flow rate of 0.5 ml/min and an injection volume of 10 μ l. An Aminex HPX-87H column (300 mm \times 7.8 mm, 5 μ m) was used for determining the glucose, xylose, and ethanol concentrations. A refractive index detector (LC 1100, Agilent, USA) was used to quantify the products.

3. RESULTS and DISCUSSION

3.1. Effects of independent variables on chemical composition

Glucan, xylan, Klason lignin and acid soluble lignin content of the solid fractions for all treatment combinations are presented in Table 2. Organosolv pretreatment with SA degraded much more than xylan and lignin, and therefore glucan content was relatively increased. All of the independent variables caused degradation of the raw materials. However, higher reaction temperature greatly increased the degradation of xylan and lignin. Run #10, pretreatment at 160°C with 1% SA for 10 min, degraded more than 90% of the xylan and Klason lignin with only 10% glucan degradation. Alternatively, Run #8, pretreatment at 152°C with 1.6% SA for 16 min, degraded the most glucan (20.57%) with

94.66% and 89.35% degradation of xylan and Klason lignin, respectively. Additionally, Run #13, pretreatment at 140°C without SA for 10 min, did not degrade the raw materials. This suggests that in organosolv pretreatment, reaction temperature is the most significant variable to degrade xylan and lignin under the presence of an acid catalyst. Although SA was not introduced in Run #13, higher reaction temperature and acetic acid auto-generated by acetyl group might be resulting slightly higher ethanol yield compared to Run #3, #5, and #9. The WIS recovery rate ranged from 37.00% to 97.11% based on treatment combinations (Table 2). The WIS recovery rate was significantly affected by the reaction temperature, followed by the SA concentration and reaction time. Compared to a recent study reporting 78.3% solid recovery for organosolv pretreatment with 1% SA of yellow poplar at 120°C, and 60.7% at 140°C (Koo *et al.*, 2011), a lower WIS recovery rate was obtained with higher lignin degradation. A higher WIS recovery rate was obtained using organosolv pretreatment with alkali catalyst to reduce lignin degradation compared to pretreatment with an acid catalyst (Koo *et al.*, 2011), illustrating the dependence of the process on the choice of catalyst. Delignification in the organosolv pretreatment is not the major factor enhancing enzymatic hydrolysis (Teramoto *et al.*, 2008). However, in this study ethanol production increased with the decrease in the lignin content of the solid fraction after pretreatment, and further study was required to reveal the action of the remaining

lignin in enzymatic hydrolysis or fermentation.

3.2. Response surface methodology for theoretical ethanol yield

The ethanol concentrations obtained after the SSF process are presented in Table 2. Most of the hydrolyzed glucose was converted to ethanol after 72 h. As expected, ethanol concentration was significantly affected by the treatment conditions and increased with the decrease in lignin content and WIS recovery rate after pretreatment. Run #8, pretreatment at 152°C with 1.6% SA for 16 min, produced the most ethanol (42.80 g/l) compared to 2.36 g/l ethanol from the control. Ethanol concentration was significantly enhanced by acid catalyst (Koo *et al.*, 2011) compared to ethanol produced from yellow poplar by organosolv pretreatment with an alkali catalyst (29.9 g/l, 140 °C with 1 % sodium hydroxide for 10 min).

To confirm the efficiency of the pretreatment and SSF process, the theoretical ethanol yield based on the glucose content in a pretreated solid fraction (Y_1) was calculated with Eq. (2):

$$Y_1(\%) = \frac{Conc_{ethanol}}{G \times K \times (180/162) \times 0.51} \times 100 \quad (2)$$

where $Conc_{ethanol}$ is the concentration of produced ethanol (g/l), G is the glucan amount in the pretreated solid fraction, K is the consistency for SSF (100 g/l), 180/162 is the conversion constant for glucan to glucose and 0.51 is the conversion constant for glucose to ethanol.

Table 3. Analysis of variance for fitted model for theoretical ethanol yield

| Source | df | Sum of squares | Mean squares | F value | P value | R ² /Adj R ² /Pred R ² | CV(%) / Adeq precision |
|---------------------------------|----|----------------|--------------|---------|----------|---|------------------------|
| Regression | 7 | 8604.81 | 1229.26 | 18.03 | 0.0001 | 0.93/0.88/0.70 | 11.59/13.02 |
| x ₁ | 1 | 5217.49 | 5217.49 | 76.51 | < 0.0001 | | |
| x ₂ | 1 | 788.66 | 788.66 | 11.57 | 0.0079 | | |
| x ₃ | 1 | 1295.86 | 1295.86 | 19.00 | 0.0018 | | |
| x ₁ * x ₂ | 1 | 180.77 | 180.77 | 2.65 | 0.1379 | | |
| x ₁ * x ₃ | 1 | 196.06 | 196.06 | 2.88 | 0.1242 | | |
| x ₁ ² | 1 | 767.55 | 767.55 | 11.26 | 0.0085 | | |
| x ₃ ² | 1 | 344.95 | 344.95 | 5.06 | 0.0511 | | |
| Lack of fit | 7 | 607.00 | 86.71 | 25.88 | 0.0377 | | |
| Pure error | 2 | 6.70 | 3.35 | | | | |
| Residual | 9 | 613.70 | 68.19 | | | | |
| Total | 16 | 9218.51 | | | | | |

A maximum theoretical ethanol yield was 98.76% from Run #8 (152 °C with 1.6% SA for 16 min), and greater than 80% theoretical ethanol yield was obtained from over 140 °C reaction temperature. These results corroborate those of a previous investigation in which the enzymatic conversion rate of a pretreated lignocellulosic biomass by organosolv pretreatment with an acid catalyst was significantly enhanced by increasing the pretreatment temperature (Zhao *et al.*, 2009).

RSM was used to determine the optimal pretreatment conditions to produce the greatest theoretical ethanol yield, and the modeling results are presented in Fig. 1 and Eq. (3). In order to visualize the first order interaction effect for theoretical ethanol yield, three dimensional plot and two dimensional contour are shown in Fig. 1a and b. z_1 is the predicted theoretical ethanol yield (%) and the analysis of the variance for the fitted model is presented in Table 3.

$$z_1 = 81.81 + 19.55x_1 + 7.60x_2 + 9.74x_3 - 4.75x_1x_2 - 4.95x_1x_3 - 7.88x_1^2 - 5.28x_3^2 \quad (3)$$

This regression model was significant at a 99% confidence level ($p = 0.0001$), and the P-values of the reaction temperature ($p < 0.0001$), reaction time ($p = 0.0079$), and SA concentration ($p = 0.0018$) were less than 0.01. Of the independent variables considered, the reaction temperature had a significant influence on the theoretical ethanol yield, followed by SA concentration and reaction time. As observed from Table 3, the reaction temperature, reaction time and SA concentration all had positive influences on ethanol yield. However, interactions between the reaction temperature and reaction time, reaction temperature and SA concentration, and quadratic terms of the reaction temperature, reaction time and SA concentration had negative influences on the theoretical ethanol yield, although the interactions were not

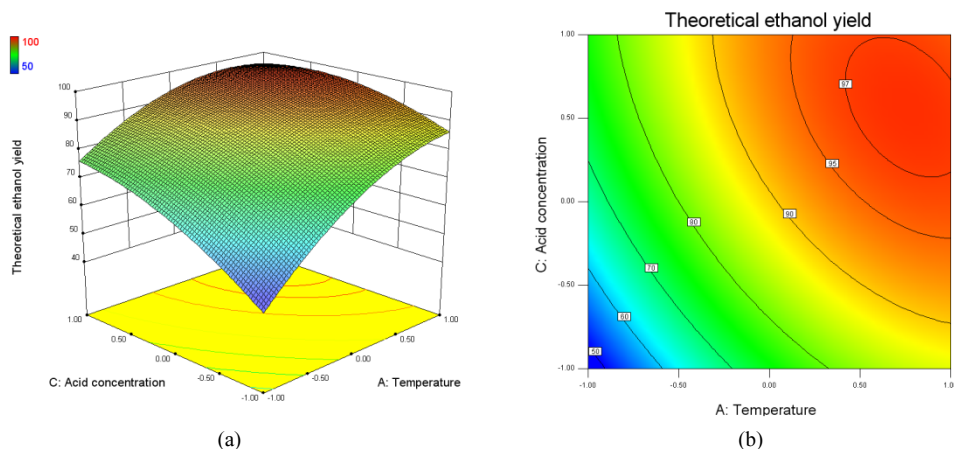


Fig. 1. Effect of independent variables interaction (a: three dimensional plot, b: detailed contour) on theoretical ethanol yield. Reaction time was fixed at 20 min (coded level: - 1.68).

significant at $p < 0.05$.

3.3. Response surface methodology for overall ethanol yield

To identify the productivity of ethanol from the organosolv pretreatment and SSF process, the overall ethanol yield based on the initial input (Y_2) was calculated using Eq. (4):

$$Y_2(\%) = \frac{\text{Conc}_{\text{ethanol}} \times \text{WIS}_{\text{recovery}}}{K} \quad (4)$$

where $\text{Conc}_{\text{ethanol}}$ is the concentration of produced ethanol (g/l), $\text{WIS}_{\text{recovery}}$ is the water insoluble solid recovery rate (%) and K is the consistency for SSF (100 g/l).

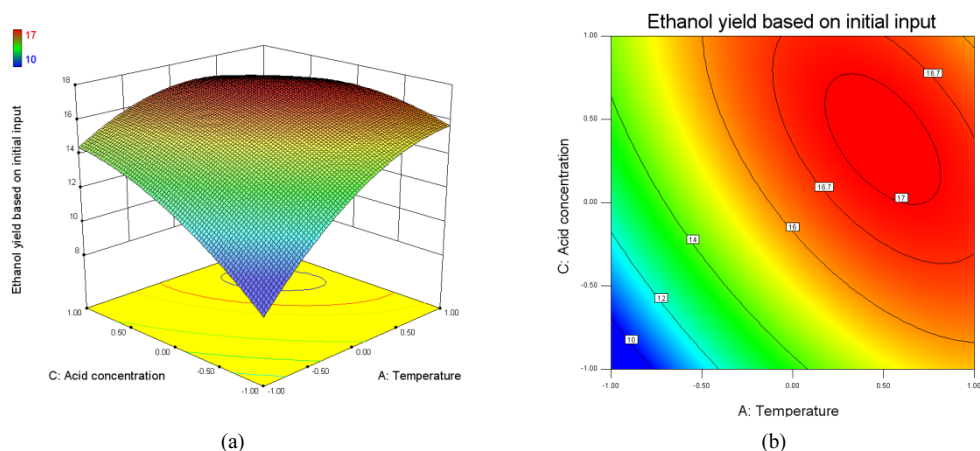
The maximum overall ethanol yield was 16.46% from Run #2 (152°C with 0.4% SA for 4 min). Additionally, Run #4 (152°C with 0.4 % SA for 16 min) and Run #6 (152°C with 1.6 % SA for 4 min) provided greater than 16%

ethanol yield. The highest theoretical ethanol yield was obtained from Run #8 (152°C with 1.6% SA for 16 min). However, because of the poor WIS recovery rate caused by excessive degradation of the raw material, the overall ethanol yield was slightly less than those of other pretreatment conditions. Previous research producing 13.0% overall yield of bioethanol by concentrated sulfuric acid hydrolysis from glucose of cornstalks was significantly less than that of this study (Demirbas, 2005). Furthermore, considering the possibility of xylose fermentation, the overall yields of bioethanol by organosolv pretreatment with SA might be greater.

The modeling results for overall ethanol yield are illustrated in Fig. 2 and Eq. (5). The first order interaction effects on the overall ethanol yield are shown in Fig. 2a and b. z_2 is the predicted overall ethanol yield (%). ANOVA for the fitted model is presented in Table 4.

Table 4. Analysis of variance for fitted model for overall ethanol yield

| Source | df | Sum of squares | Mean squares | F value | P value | R ² /Adj R ² /Pred R ² | CV(%)/Adeq precision |
|---------------------------------|----|----------------|--------------|---------|----------|---|----------------------|
| Regression | 7 | 266.22 | 38.03 | 21.59 | < 0.0001 | 0.94/0.90/0.76 | 10.24/14.62 |
| x ₁ | 1 | 149.71 | 149.71 | 84.99 | < 0.0001 | | |
| x ₂ | 1 | 19.60 | 19.60 | 11.13 | 0.0087 | | |
| x ₃ | 1 | 31.41 | 31.41 | 17.83 | 0.0022 | | |
| x ₁ * x ₂ | 1 | 11.05 | 11.05 | 6.27 | 0.0336 | | |
| x ₁ * x ₃ | 1 | 16.31 | 16.31 | 9.26 | 0.0140 | | |
| x ₁ ² | 1 | 31.84 | 31.84 | 18.07 | 0.0021 | | |
| x ₃ ² | 1 | 13.92 | 13.92 | 7.90 | 0.0203 | | |
| Lack of fit | 7 | 15.74 | 2.25 | 39.26 | 0.0251 | | |
| Pure error | 2 | 0.11 | 0.06 | | | | |
| Residual | 9 | 15.85 | 1.76 | | | | |
| Total | 16 | 282.07 | | | | | |

**Fig. 2.** Effect of independent variables interaction (a: three dimensional plot, b: detailed contour) on overall ethanol yield. Reaction time was fixed at 20 min (coded level: - 1.68).

$$z_2 = 15.10 + 3.31x_1 + 1.20x_2 + 1.52x_3 - 1.18x_1x_2 - 1.43x_1x_3 - 1.61x_1^2 - 1.06x_3^2 \quad (5)$$

This regression model ($p < 0.0001$) and independent variables including reaction temperature ($p < 0.0001$), reaction time ($p = 0.0087$), and SA concentration ($p = 0.0022$) were sig-

nificant at a 99% confidence level. Similar to the theoretical ethanol yield, the reaction temperature, reaction time and SA concentration had significant influences on overall ethanol yield, whereas interactions between reaction temperature and reaction time, reaction temperature and SA concentration, and quadratic terms

Table 5. Solutions for optimal conditions and validation

| Solution # | Temperature (°C) | Reaction time (min) | Acid concentration (%) | Predicted yield (%) | Validated yield (%) |
|------------|------------------|---------------------|------------------------|---------------------|---------------------|
| 1 | 146.0 | 15.9 | 1.22 | 17.11 | 17.15 |
| 2 | 146.0 | 15.9 | 1.22 | 17.11 | - |
| 3 | 145.7 | 15.9 | 1.22 | 17.11 | - |
| 4 | 146.3 | 15.9 | 1.22 | 17.11 | - |
| 5 | 146.2 | 15.9 | 1.24 | 17.11 | - |
| 6 | 146.7 | 15.9 | 1.09 | 17.07 | - |
| 7 | 147.5 | 15.9 | 1.04 | 17.04 | - |
| 8 | 151.7 | 15.9 | 1.08 | 16.85 | 17.14 |
| 9 | 151.9 | 9.2 | 1.01 | 16.80 | 16.91 |
| 10 | 151.9 | 5.6 | 1.03 | 16.79 | - |

of reaction temperature, reaction time and SA concentration had negative influences on overall ethanol yield. Contrary to ANOVA results regarding theoretical ethanol yield, both interactions between the reaction temperature and reaction time ($p < 0.0336$), reaction temperature and SA concentration ($p < 0.0140$) were statistically significant.

The interaction effects of the reaction temperature and SA concentration are shown in Fig. 2. At low reaction temperatures, the overall ethanol yield rises sharply with an increase in the SA concentration. However, at high temperatures, the high SA concentration starts to negatively impact ethanol yield due to glucose loss in the pretreatment process because of rapid decomposition at high temperatures under acidic conditions (Teramoto *et al.*, 2008).

3.4. Optimization and validation

The conditions required for achieving the maximum overall ethanol yield were optimized using the obtained model and input criteria. By

considering each response value, 13 solutions were determined using Design Expert 8.0.6 software (Stat-Ease, Inc., MN, USA), and the top ten solutions are shown in Table 5. The maximum response for the quadratic model was achieved at 146°C with 1.22% SA for 15.9 min, predicting an overall ethanol yield of 17.11%. In order to confirm the predicted results, organosolv pretreatment was performed at three different optimum conditions (solution #1, 8, and 9). The actual optimum yields of solution #1, #8, and #9 were 17.15%, 17.14% and 16.91% of the overall ethanol yield, respectively. The experimental values were very close to the predicted values, and the optimal condition was located at the central point within the experimental scale. Therefore, the quadratic model was found to be reliable.

A mass balance diagram is shown in Fig. 3. A large quantity of 17.15 g ethanol was produced from 100 g DM yellow poplar because most of the glucose was converted to ethanol. The large amount of xylose in the liquid hydrolysates should be used for fermentation after the

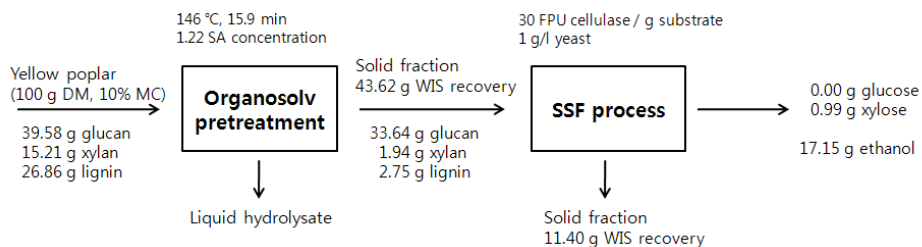


Fig. 3. Mass balance diagram of organosolv pretreatment followed by SSF process.

detoxification process. Furthermore, the organosolv lignin dissolved in liquid hydrolysates and the enzymatic hydrolyzed lignin remaining after the SSF are likely to be of large amount without remarkable denaturalization, suggesting added value as an additional products that may be used as structural material.

4. CONCLUSION

In organosolv pretreatment, reaction temperature has the most influence of all variables on the degradation of xylan and lignin. The highest theoretical ethanol yield (98.76%) was obtained at 152 °C with 1.6% SA for 16 min, however the overall ethanol yield was decreased due to low WIS recovery rates. The optimal conditions for maximum overall ethanol yield were determined (146 °C with 1.22 % SA for 15.9 min) with a predicted yield of 17.11%. The experimental values (17.15%) were very close to the predicted values, and the optimal condition was located at the central point, indicating that the quadratic model was reliable.

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