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Fractionation of barley straw with dilute sulfuric acid for improving hemicellulose recovery

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Abstract−Dilute acid fractionation of barley straw improves dissolving hemicellulose fraction of the straw, while leaving the cellulose more reactive and accessible to enzyme as a strategy of pretreatment. To characterize the fractionation process, the effects of the acid concentration, temperature and reaction time on the hemicellulose removal as well as on the formation of by-products (furfural, 5-hydroxymethylfurfural and acetic acid) were investigated. The optimum fractionation conditions of barley straw were 1% (w/v) concentration of sulfuric acid, 158 °C of reaction temperature and 15 min of reaction time. Under the optimum conditions, 87% of xylan was hydrolyzed and recovered in liquid hydrolyzate, which was 7% higher than that of the predicted yield. The hydrolyzate contained glucose 2.44 g/L, arabinose 1.70 g/L, xylose 13.41 g/L, acetic acid 1.55 g/L, levulinic acid 0.03 g/L, 5-HMF 0.03 g/L and furfural 0.75 g/L.

Key words: Barley Straw, Response Surface Methodology (RSM), Pretreatment, Hemicellulose, Sugar Decompositions

INTRODUCTION

Leading nations have set a goal of making biofuel constitute 25% of global energy within 20 years. The United States is diversifying its energy supply realizing the urgency of technological innovation in the production of biofuel [1]. In pursuing the development of bioenergy, nations must be careful not to allow fuel to infringe upon economic supply. Currently, alternative farm products, such as rice straw, and barley straw, are processed after grain harvesting to create biofuel. Korean farm products such as barley straw, rapeseed straw, rice straw, pepper stem, etc. are highly valued for biofuel production. There is high potential for biofuel as feedstock; however, 80% of such biofuel resources are not recycled. By tapping into renewable resources, an alternative plan can be established which utilizes Korea's natural wealth. Barley straw has the potential to serve as a low cost, renewable organic substance for ethanol production. Lignocellulosic biomass is the most abundant organic material on earth and is also a promising raw material for bioenergy production [2,3]. The principal components of lignocellulosic biomass are cellulose (30-40%) and hemicellulose (20-25%), which are bound together by lignin (20-25%) [4,5]. Many physicochemical structural and compositional factors of the straw hinder the hydrolysis of cellulose present in biomass to sugars and other organic compounds that can later be converted to fuels. To overcome the difficulties regarding the recalcitrance of the biomass, improvement of the pretreatment technique and enzymatic hydrolysis steps is crucial. The bottleneck in the commercial use of lignocelluloses in ethanol production lies in the hydrolysis step due to its high enzyme requirements and, therefore, enzyme cost. Difficult hydrolysis of lignocelluloses is due to (a) the crystalline and complex structure of the holocellulose components of the biomass, (b) enzyme deactivation due to irreversible binding to substrates and non-productive components of biomass such as lignin, (c) enzyme inhibition due to the formation of sugar products during enzymatic reactions, and (d) recalcitrant structure of the lignocelluloses [5-9].

Biomass conversion technologies, based on use of waste or residues as feedstock for biofuels production, has emerged to accommodate the new feedstock. While the production of bioethanol from sugars and starch is more straightforward, bioethanol production from lignocellulosic biomass creates additional technical challenges, such as a need for chemical/physical pretreatment to break up the biomass structure and facilitate enzyme hydrolysis [11-14]. In recent years, hydrothermal fractionations, including steam explosion, hot water, and dilute-acid fractionation, have been extensively studied and reported in the literature and typically employ dilute acid to hydrolyze the hemicellulose [3,11-21]. After the dilute acid fractionation with sulfuric-acid was performed, the lignocellulosic biomass released xylose effectively and was successfully recovered.

In this work, the dilute-acid fractionation of barley straw was optimized through the utilization of statistical experimental design. Evaluation criteria for optimization of the fractionation conditions were based on high xylose recovery and low inhibitor contents in the hydrolyzates. In addition, this paper reports the compositional analysis of hydrolyzate liquors and solids, xylose and glucose mass balance closures, and digestibility of the acid fractionated barley straw. The treated samples were further analyzed with SEM and XRD in order to determine optimal fractionation conditions. The purpose of this study was to gain a more accurate understanding of the quantities of acid required for effective fractionation and the reactivity trade-offs with reaction time and temperature that will enable overall process optimization.

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MATERIALS AND METHODS

1. Raw Material

Barley straw was used throughout this study and was supplied by the Bioenergy Crop Research Center, National Institute of Crop Science, Rural Department Administration (Muan, Jeollanamdo, Korea). The straw was ground to an average size of 14-45 mesh (0.35-1.41 mm) using a laboratory knife. The screened straw chips were air-dried for 24 h at 45±5 °C and then used directly in dilute-acid fractionation studies. The moisture content of milled straw was 5.17% based on total wet biomass weight.

2. Dilute Sulfuric-acid Fractionation

The fractionation experiments were performed using sealed bomb tubular reactors. The vessels were 150 mm long and 10.7 mm inside diameter and constructed out of 316 stainless steel tubing, capped at either end with Swagelok fittings, giving an internal volume of 13.5 cm^3. The stainless steel reactors were loaded with 500 mg of oven-dried barley straw. The residual moisture in the air-dried straw was accounted for in the determination of the amount of acid solution to be added, which gave a final ratio of 15 mL liquor per gram oven-dry straw. The reaction conditions tested were temperature (143 °C and 177.3 °C), concentration of acid (0.32-1.18%, w/v), and reaction time (0-30 min). The temperature, acid concentration, and residence time for the experimental design are given in Table 1 [23]. A total of 17 runs were carried out, with runs 9-11 each run three times.

Table 1. Three-variable, three-level fractional factorial design for the barley straw fractionation

# Run	Coded value			Process variable		
	X_1	X_2	X_3	T (°C)	AC (%)	t (min)
1	1	1	0	170	1	10
2	1	0	1	170	0.75	15
3	1	−1	−1	170	0.5	5
4	0	1	−1	160	1	5
5	0	−1	1	160	0.75	15
6	−1	1	1	150	1	15
7	−1	−1	0	150	0.5	10
8	−1	0	−1	150	0.75	5
9	0	0	0	160	0.75	10
10	0	0	0	160	0.75	10
11	0	0	0	160	0.75	10
12	1.732	0	0	177.3	0.75	10
13	−1.732	0	0	143	0.75	10
14	0	1.732	0	160	1.18	10
15	0	−1.732	0	160	0.32	10
16	0	0	1.732	160	0.75	18.6
17	0	0	−1.732	160	0.75	1.34

T=temperature, AC=acid concentration (w/v), t=reaction time, X_1=(T-160)/10, X_2=(AC-0.75)/0.25, and X_3=(t-10)/5

3. Chemical Analysis

The composition element of barley straw was analyzed by the NREL Chemical Analysis and Testing Standard Procedures [20]. And determination of the structural carbohydrates, lignin, and moisture contents of the starting biomass materials followed standard analytical methods established by the National Renewable Energy Laboratory (NREL) [22]. The hydrolyzates and fermentation inhibitors were analyzed by HPLC. The Breeze system (Waters Co., Milford, MA, USA) used for carbohydrate measurement had a Bio-Rad Aminex HPX-87H column (300 mm×7.8 mm) and Cation H micro-guard cartridge (30×4.6 mm; Bio-Rad Laboratories Inc., Hercules, CA). The column was maintained at 60 °C, with a 5 mM H_2SO_4 effluent at a flow rate of 0.6 mL/min. All of the sugar peaks detected by a refractive index detector (Waters 2414, Waters Co., Milford, MA, USA) were identified and quantified by comparison to the retention times of authentic standards. The Bio-Rad Aminex HPX-87H analytical column allows for the concurrent analysis of a liquid sample for the presence of acetic and formic acid as well as sugar degradation products. Quantification of the samples was calculated by HPLC from the peak areas of the standard solutions for three sugars: D (+)-glucose, D (+)-arabinose, and D (+)-xylose.

RESULTS AND DISCUSSION

1. Composition of Fractionated Barley Straw

Table 2 shows the analysis results of the material (based on a 105 °C dry weight). The initial composition of barley straw was determined as: 37.8% glucan, 21.5% xylan, and 2.5% arabinan. Carbohydrates accounted for 61.8% of the dry material, which makes barley straw a very promising lignocellulosic biomass for ethanol production. The composition of non-carbohydrate is 18.9% lignin, 9.2% ethanol (EtOH) extractive, 1.8% hexane extractive, 2.8% protein, 2.6% ash, and 1.1% others.

2. Optimization of the Fractionation Conditions

2-1. Influence of Solid/Liquid (S/L) Ratio on Xylose Recovery

An optimized ratio of solid to liquid (S/L) is required for maximum xylose recovery. In this experiment, the ratio of substrate and acid liquid mixture in the reactor determined by the S/L ratio followed fractionation yield, as compared to when the dilute acid fractionation process progressed to find the most suitable S/L ratio. Fig. 1 presents the effects of the mass ratio (substrate: reagent) on xylose recovery in dilute acid fractionation for 15 min with 1% (w/v) acid concentration at 158 °C. A significant amount of xylose was recovered into hydrolyzate in response to increasing the mass portion of

Table 2. Chemical components of barley straw on dry basis

Glucan (%)	Xylan (%)	Arabinan (%)	AIL[a] (%)	Extractive (%)		Crude[b] protein (%)	Ash (%)	Total (%)
				Ext. (EtOH)	Ext. (Hexane)			
37.8±0.61	21.5±0.29	2.5±0.05	18.9±1.08	9.2±0.18	1.8±0.08	2.8±0.28	2.6±0.21	97.2±2.78

[a] Acid insoluble lignin

[b] (%) Crude protein=(%) nitrogen×6.25 (nitrogen factor)

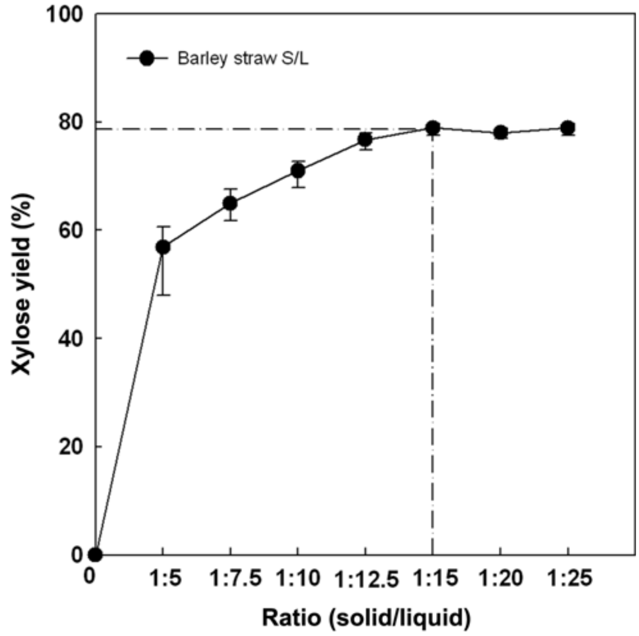


Fig. 1. Comparison of recovery yield for xylose from hydrolyzate as a function of different ratio of solid to liquor.

the reagent. Approximately 80.0% of xylose was dissolved into the hydrolyzate with a mass ratio of 1 : 15. The recovery yield of xylose improved very little when the ratio of substrate to reagent rose above 1 : 15. The results appear to show that the highest xylose recovery performance can be achieved for the dilute acid fractionation with the ratio of 1 : 15.

2-2. Process Variables and Inhibitor Compounds

Fig. 2 summarizes the xylose yields obtained during the fractionation at three different temperatures (140 °C, 150 °C, and 160 °C) with various acid concentrations (0.5-1.25% (w/v)). For each combination of temperature and several reaction times, acid concentration was studied ranging from 0.5% to 1.25% (w/v). As shown in Fig. 2(a), the reactions occurring at 140 °C showed that the rate of xylose recovery yield was continuously increasing over the acid concentration and reaction time. In the reaction at 150 °C and 160 °C, a maximum of 85.5% and 84.2% of the xylose was found in the hydrolyzate. The maximum occurred at an isothermal reaction time of 15 min. Many decomposition products generated under the reaction conditions are also shown in Fig. 3. With the reaction temperature increasing, the production of sugar decomposed increased. The acetate formation appears to be maximum at a temperature between 150 and 160 °C for the residence time. During hemicellulose dissolution, in addition to carbohydrates, water soluble lignocellulosic degradation products were also liberated (Fig. 3). Most of these compounds are potential inhibitors of enzymes and fermenting organisms. Analysis of these inhibitory compounds (acetic acid, formic acid, furfural, 5-HMF, and levulinic acid) was conducted on the liquid hydrolyzate derived from dilute acid fractionation of the barley straw. Acetic acid, formic acid, and furfural constituted most (70-80%) of the total potential inhibitory compounds, while lignin degradation products constituted only about 10-20% (data not shown) in all the samples. This clearly indicates that pentose sugars (xylose and arabinose) derived from hemicellulose fraction

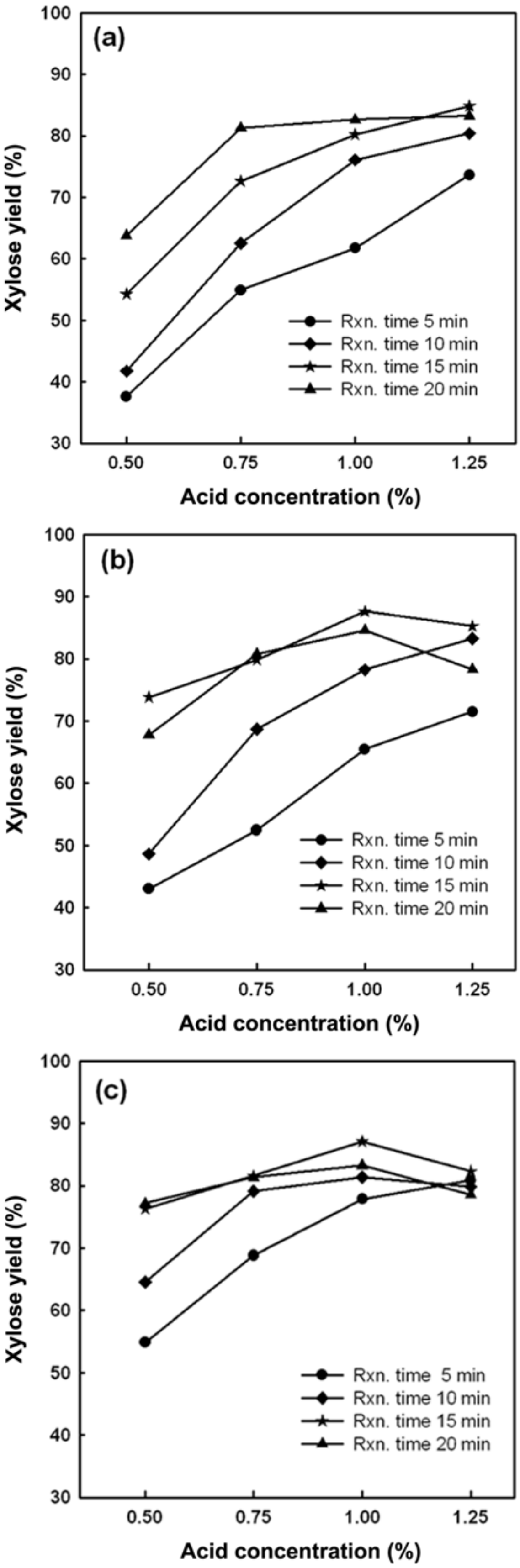


Fig. 2. Xylose yield from acid fractionation of barley straw as a function of various reaction conditions: (a) 140 °C (b) 150 °C (c) 160 °C.

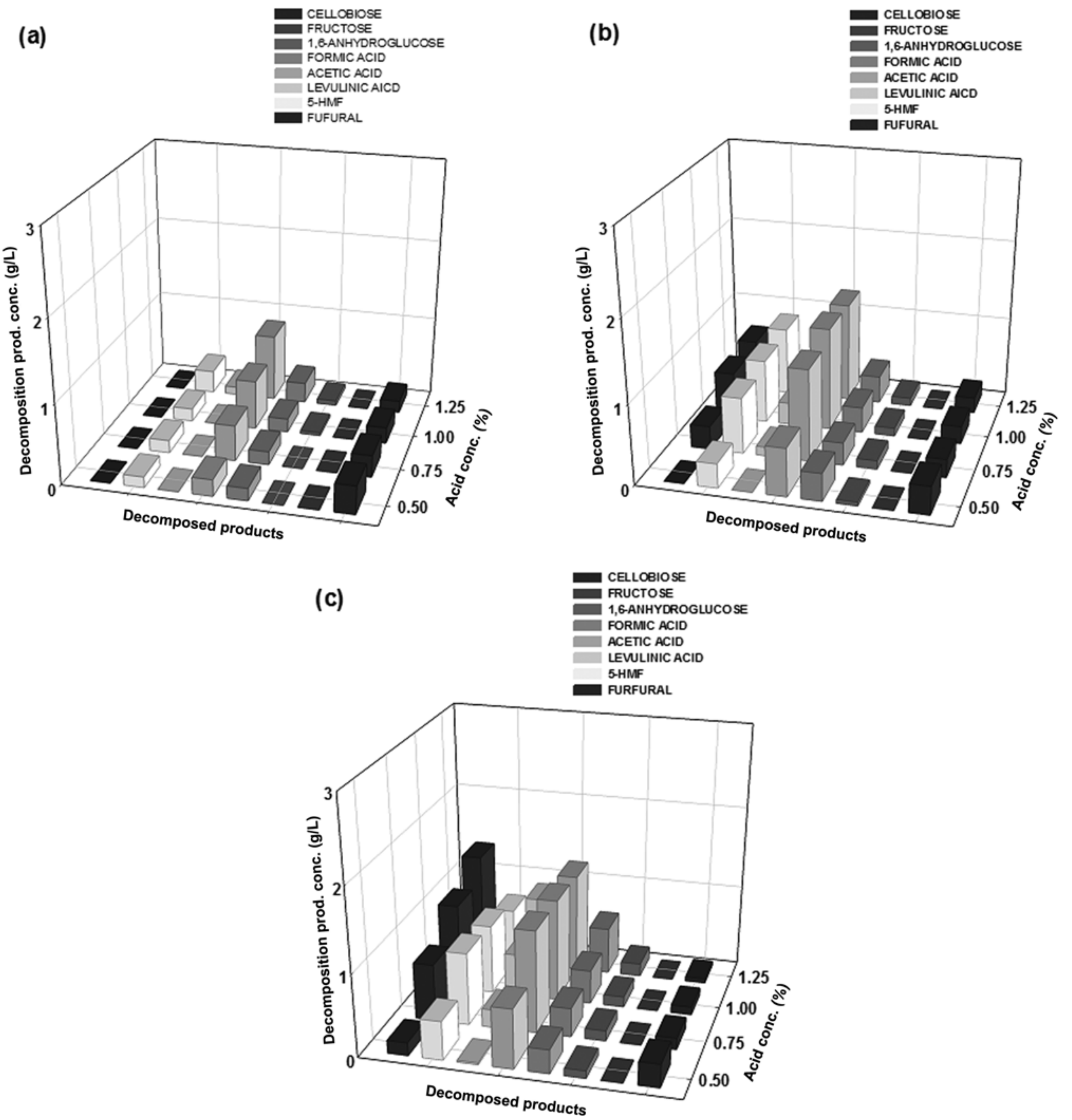


Fig. 3. Comparison of inhibitor levels in process hydrolyzates of acid fractionated barley straw under various reaction conditions: (a) 140 °C (b) 150 °C (c) 160 °C.

were further degraded.

2-3. Optimization of Dilute Acid Fractionation Process of Barley Straw with RSM

The experiment model's linear regression coefficient of determination (R^2) of this experiment was 0.80. These conditions, reaction temperature (X_1), acid concentration (X_2), and reaction time (X_3), at maximum yield of xylose can be determined from Table 1. Fig. 4 presents the results of a series of dilute-acid fractionation evaluated through a 3^3 full factorial design. According to RSM, the optimum temperature, acid concentrations, and residence time were determined. The graphical representation provides a method for visualizing the relationship between the response and the interactions among test variables in order to determine the optimum conditions (Fig. 4). The predicted fermentable xylose achieved from this simulation was 79.9% of the maximum possible yield at 158 °C, 1.0% and reaction time of 15 min. The experiment confirmed the xylose yield was obtained with 87.0% under the optimum conditions. The value was approximately 7% higher than that of the predicted yield. Fig. 5 presents the result of an acid fractionation of barley straw that was carried out under the optimum operating conditions predicted by

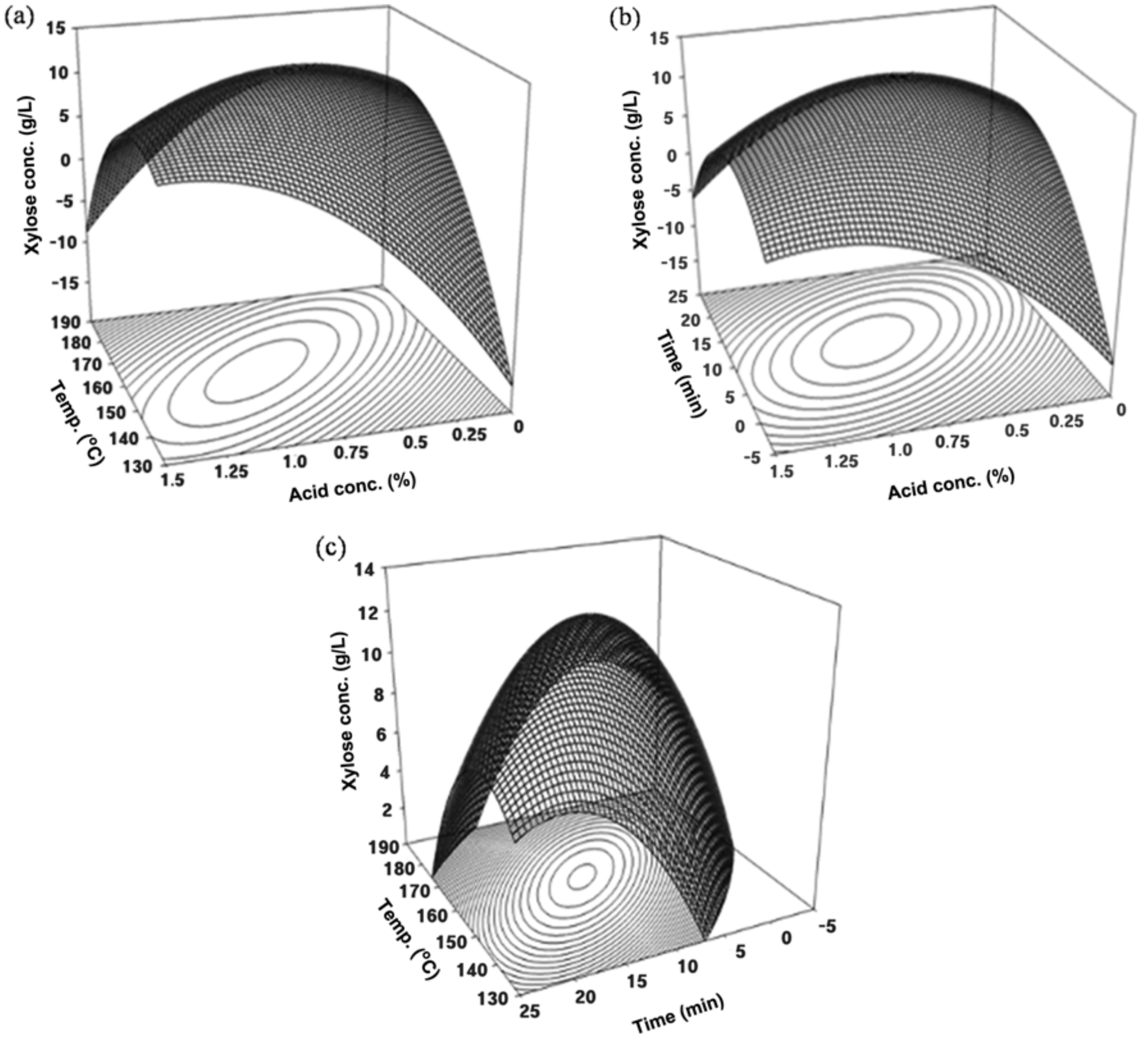


Fig. 4. Response surface and contour lines combined effects of (a) temperature and acid concentration at a reaction time of 15 min, (b) reaction time and acid concentration at a temperature of 158 °C, and (c) temperature and reaction time with 1% (w/v) acid concentration.

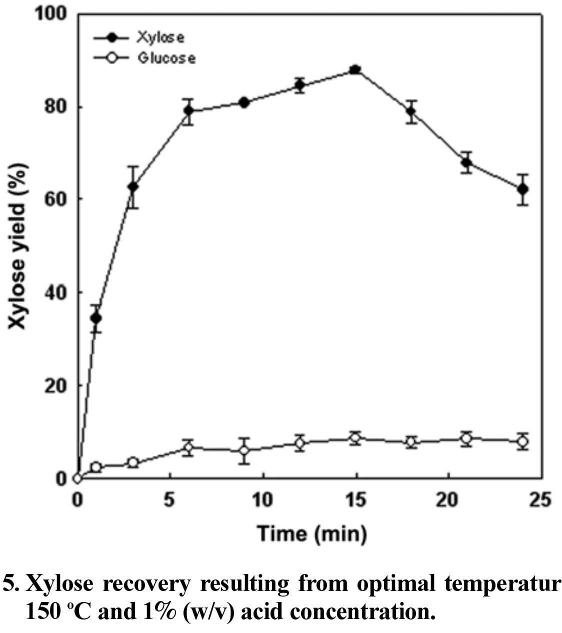


Fig. 5. Xylose recovery resulting from optimal temperature of 150 °C and 1% (w/v) acid concentration.

the RSM method.

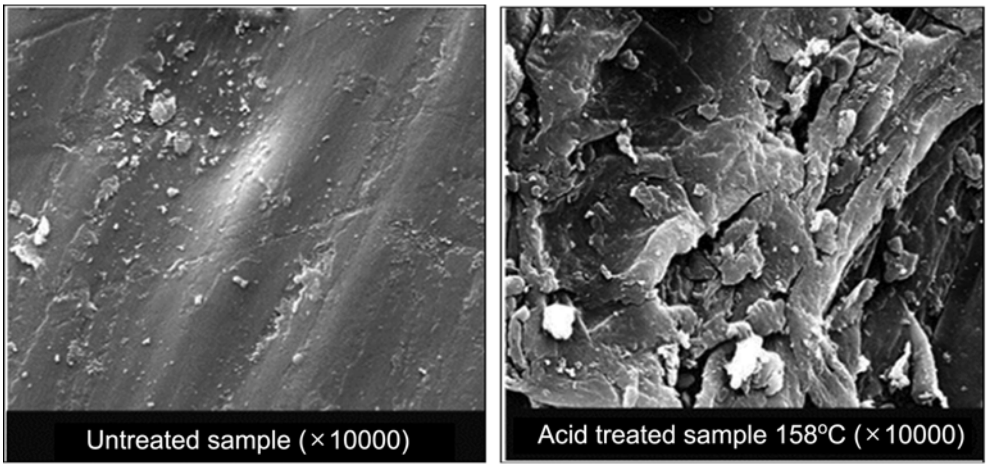
2-4. Image Analysis of Fractionated Barley Straw

Fig. 6 illustrates an SEM(Quanta200, FEI, USA) image taken on a fractionated straw's surfaces. The appearance of ridge-like features was attributed to the preferential degradation of the more labile components of biomass such as hemicellulose and cellulose. Meanwhile, these features are not visible in the SEM micrograph of untreated straw despite its higher magnification (Fig. 6(a), (b)).

Fig. 7 shows the X-ray patterns of fractionated barley straw (XRD: MAX-2500V, Rigaku Co., Japan). The peaks in the range of $2\theta=18.7^{\circ}\sim22.5^{\circ}$ representing cellulose crystal structure were compared. The CrI (crystallinity index) of fractionated barley straw under the optimized conditions was 62.57 and is higher than that of untreated straw (CrI=38.66) by 23.91%. This means increasing purity of cellulose of barley straw by fractionation, because of removing the amorphous materials such as hemicelluloses etc.

2-5. Mass Balance Closure

Table 3 shows the results of an analysis (based on a 105 °C dry wt.) of the composition of raw barley straw, the fractionated straw,



(a) (b)

Fig. 6. SEM micrographs of the barley straw: (a) untreated straw, (b) pretreated straw.

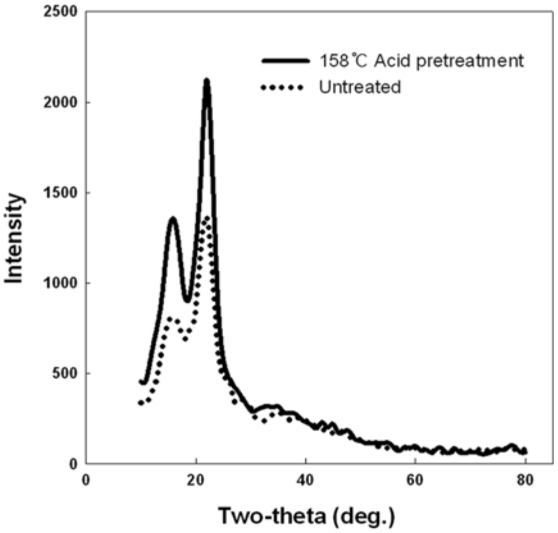


Fig. 7. Analysis of XRD (X-ray diffraction spectra): the specimen was scanned from $2\theta=10^{\circ}$ to 80° with a step size of 2°.

and the hydrolyzate. The fractionation was carried out at the optimal condition pre-determined through RSM method (1% sulfuric acid, 10 min reaction time, and a temperature of 158 °C). Approximately 47.5% of the mass of the barley straw was extracted into hydrolyzate. The total extraction mass balance of sugar was 96.7% for glucan and 94.1% for xylan. The glucan content of acid-treated straw was higher relative to the raw material. This is expected because little of the glucan is dissolved during dilute acid fractionation, whereas a much larger fraction of the hemicellulose is dissolved.

CONCLUSION

For production of bioethanol, biomass fractionation and the cost of enzymes are critical targets for process and cost improvements. As a biomass pretreatment strategy, this study predicts an optimal fractionation condition for barley straw through RSM. It was found that the efficiency of acid fractionation with 1% (w/v) acid concentration resulted in the highest recovery of xylan for 15 min at a temperature of 158 °C. Under the condition optimized, the maximum

Table 3. Mass balance in dilute acid fractionation of barley straw at optimum reaction conditions

Substrate	Weight remaining [%]	Solid [%]								Liquid [%]		EMB [%][a]	
		Glucan	Xylan	AIL[b]	Extractive		Crude protein	Ash	Total	Glucan	Xylan	Glucan	Xylan
					EtOH	Hexan							
Untreated	100	37.8	21.5	18.9	9.2	1.8	2.8	2.6	94.7	-	-	-	-
Treated	52.5	62.5	2.9	16.2	-	-	-	1.2	-	2.5	18.7	96.7 (−3.3)	94.1 (−5.9)
Treated[c]		*32.8*	*1.5*	*8.5*				0.6	-				
Component retention [%]		86.9	7.2	44.8	-	-	-	24.1	-	-	-		

[a]Extraction mass ralance= $\frac{\sum C_{Li}+\sum C_{Si}}{\sum C_{Ri}}$

Where C_i is the mass of each sugar component as determined through HPLC chromatography, the subscripts L, S and R refer to the extracted liquid, extracted solids and raw straw fractions, respectively

[b]Acid Insoluble Lignin

[c]Analysis data are based on the oven dry untreated biomass

The barley straw is pretreated for 15 min with 1.0% (w/v) H_2SO_4 at 158 °C recovery yield of xylose obtained was 87.0%. The mass balance closure for barley straw fractionation with dilute sulfuric acid was found to be close to 95%. This confirms that reported concentrations of hydrolyzate components are likely to be representative of actual mass fractionation in the process. This strongly suggests that fractionation of barley straw using suggested method prior to C5 sugar fermentation process is feasible and viable for the future biorefinery industry.

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