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Research

Conversion of Ligno Cellulose to Biofuel

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Abstract : *A two-stage process using aqueous ammonia and hot-water has been investigated to fractionate corn stover. To optimize the fractionation process so that hemicellulose recovery and purity in the liquid hydrolysate were maximized, the experiments were carried out employing response surface methodology (RSM). A central composite design (CCD) was used to evaluate and confirm the effectiveness and interactions of factors. The optimal fractionation conditions were determined to be as follow: (1) First-stage reactor operated in batch mode using a 15% NH_4OH solution (W_{NH3} =15%) at 1:10 solid:liquid ratio, 60 °C and 24 h; (2) second stage percolation reactor operated using hot-water at 20 ml/min, 200 °C, and 10 min. The model predicted 51.5% xylan recovery yield and 82.4% xylan purity under these conditions. Experiments confirmed the maximum xylan recovery yield and purity were 54.7% and 83.9% respectively under the optimal reaction conditions.*

Keywords: *Corn stover; Biorefinery; Value-added co-products; Simultaneous Saccharification and Fermentation (SSF); Xylooligomer; Ethanol; Zea mays*

Introduction

Perhaps the most serious issue confronting the world is the vitality emergency. The cost of oil significantly raised in 1973, making an oil emergency. Vitality utilization has expanded relentlessly as the total populace has developed and more nations have turned out to be industrialized. The petroleum products, including unrefined petroleum, coal and flammable gas are the significant assets to satisfy the expanded vitality needs. As generally accepted, the fossil energies will be depleted soon (Wyman, 2001). The world right now expends 30 billion barrels of

oil for each year; Colin (2003) gauges that oil stores will turn out to be rare by the 2050s. Since oil is a nonrenewable asset, there is a dire need to look for elective vitality sources that are limitless. In late decades, a worldwide consciousness of the expanding CO_2 fixation levels in the environment and worry for an Earth-wide temperature boost prompted the plan of the Kyoto Protocol in 1997 which has driven numerous nations to make the duty to diminish the outflow of CO_2. One method for diminishing CO_2 discharges could be substitution of petroleum derivatives with sustainable power sources.

The improvement of elective fuel and vitality sources has in this way turned into an overall research need as of late. Biofuels are powers delivered from biomass. These powers are for the most part as bioalcohols, biodiesel, biogas and different synthetic compounds delivered from biomass. The two primary biofuels are biodiesel and bioethanol. Among those, bioethanol created by bioconversion of lignocellosic biomass is being viewed as one of the most encouraging option biofuels. The decision of lignocellulose to bioethanol transformation ought to be settled based on generally speaking financial aspects (most minimal cost), condition (least poisons) and vitality (higher productivity), i.e., exhaustive procedure advancement and streamlining are as yet required to make the procedure monetarily practical. The expanding oil cost and negative effect of non-renewable energy sources on the earth are empowering the utilization of lignocellulosic materials to help address vitality issues (Di Nasso et al., 2011).

There are numerous favorable circumstances of biofuels over petroleum derivatives that make the elective fuel source an appealing choice now and later on. The principle bit of leeway of biofuels is that they are considered 'carbon unbiased' by certain individuals. This is on the grounds that the carbon dioxide discharged during the ignition of biofuels is equivalent to the sum that absorbed during photosynthesis (Kheshgi et al., 2000) bringing about no net increment to CO2 levels. In this manner, they don't add to a worldwide temperature alteration. Utilization of biofuels discharges no sulfur and has much lower particulate and poisonous outflows, especially when contrasted and other fluid transportation fills (Scott and Wyman, 2004). Bioethanol generation can give an appealing course to discard dangerous lignocellosic squanders, for example, stalks, stovers and leaves of farming yields. Biofuels are sustainable power source (produced using natural materials and even natural waste, there is for all intents and purposes an endless measure of biofuels accessible), cheap to deliver and decrease reliance on remote oils.

Ethanol is one of the most encouraging biofuels that can be utilized to substitute fuel for tomorrow's transportation vehicles. Fuel ethanol is mostly utilized as an oxygenated fuel added substance. The higher octane number of the fuel blend, when it contains ethanol, lessens the requirement for poisonous, octane-upgrading added substances, for example, methyl tertiary butyl ether. Because of the oxygen in ethanol particles, there is likewise a decrease of carbon monoxide discharge and non-combusted hydrocarbons (Hu et al., 2008).

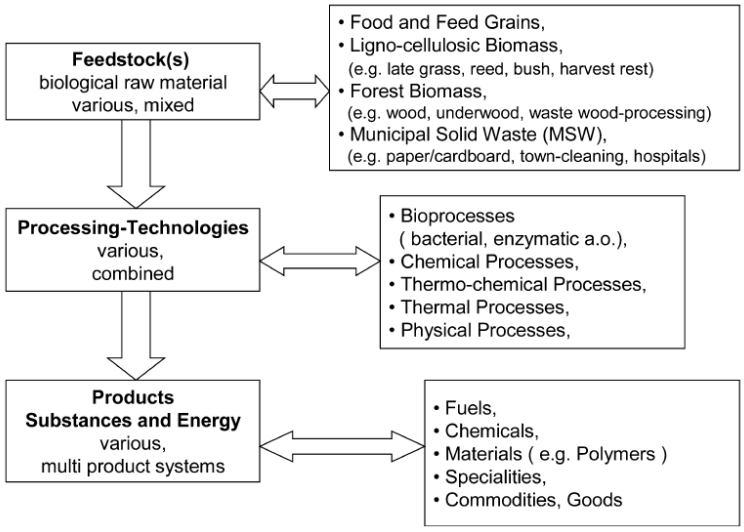

Fig. 1. Basic principles of a biorefinery (Kamm and Kamm, 2004)

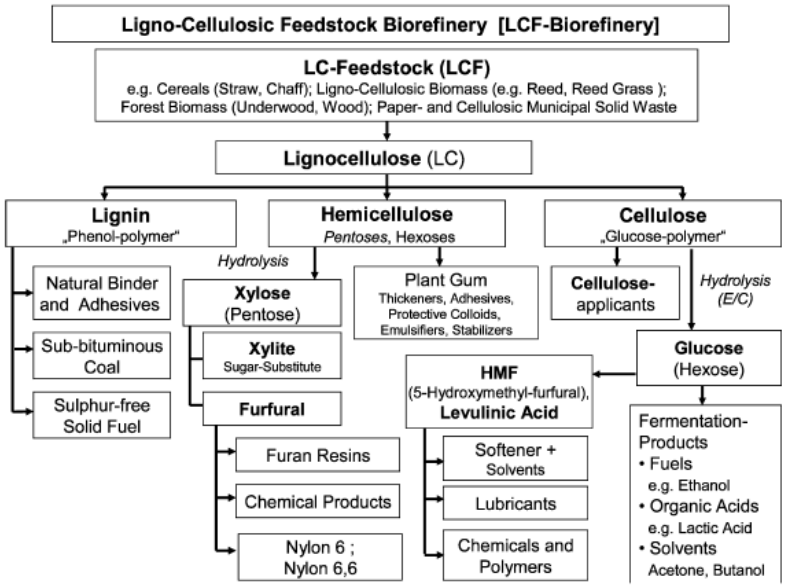

Fig. 2. Lignocellulosic feedstock biorefinery (Kamm and Kamm, 2004; Van Dyne et al, 1999)

2. Methods

2.1. Materials

Air-dried ground corn stover was supplied by the National Renewable Energy Laboratory (NREL, Golden, CO). The corn stover (*Zea mays*) which includes stalks, leaves, tassel, husks, and cobs from Pioneer 34M95 was harvested in Wray, northeastern Colorado in 2002. The harvested corn stover was washed by distilled water and air-dried at ambient temperature, and then screened to a nominal size of 9-35 mesh. The prepared corn stover was stored in the refrigerator at 4ºC. The composition of corn stover was determined by our lab following the chemical analysis and testing standard method developed by NREL [31]. The mass fraction of each component in the untreated corn stover was 34.2 % glucan, 22.3 % xylan, 1.6 % galactan, 3.1 % arabinan, 12.2 % lignin (acid insoluble + acid soluble), 3.9 % acetate, 6.2 % sucrose, 1.6 % protein, 4.0 % uronic acid, 1.2 % ash, and 10.7 % other extractives. Cellulase enzyme, GC 220 (Genencor International Inc., Lot No #301-04232-162) and Multifect-Xylanase (Genencor International Inc., Lot. #301-04021-015) were provided by Genencor International. The average activities of cellulase (GC-220) and xylanase (Multifect) were 45 FPU/ml and 8000 GXU/ml, respectively. The β-glucosidase enzyme, Novozyme 188 (Novo Inc., lot no. 11K1088), was purchased from Sigma-Aldrich (St. Louis, MO). Activity of Novozyme 188 was 750 cellobiase unit (CBU)/ml. The microorganism used for SSF was *Saccharomyces cerevisiae* ATCC® 200062 (NREL-D5A), which is a SERI strain genetically improved from Red Star baker's yeast. The growth media was YP medium. The mass fractions of yeast extract (Sigma cat. No. Y-0500) and peptone (Sigma cat. No. P-6588) in YP medium were 10 and 20 g/l respectively.

2.2. Experimental setup and Operation

Corn stover was treated with 15% NH4OH solution (WNH3 =15%) in glass media bottles (Fischer Cat# 06-414-1C) at 60 °C for 24 h. Solid-to-liquid ratio was kept at 1:10. The source of ammonia was 29.5% of ammonium hydroxide (Fisher Cat# A669C). This was diluted to 15% NH4OH solution (WNH3 =15%) with deionized (DI) water and used for the treatment. After the completion of treatment, the solids and liquids were separated by fluted filter paper (Fisher Cat# 09-790-14F), and solids were washed with DI water using vacuum filter until the wash water had a neutral pH. Solid cakes were dried in the air until the moisture content of samples reached approximately 10% (drying conditions: ambient temperature and 48-72 h of drying time) and stored in the refrigerator for the second-stage hot water treatment.

2.2.2. Second stage: hot-water treatments

The reactor system for the second stage treatment consists of a flow-through column reactor with preheating coil, an HPLC (high performance liquid chromatography) pump
(Series II pump, Chrom Tech, Inc., MN), a temperature-programmable GC (gas chromatography) oven (Hewlett Packard 5890, HP Inc., Ontario, Canada), solution reservoirs and a sample cylinder tank. The reactor (70 ml of internal volume) was constructed from a 16.5 cm length of 2.3 cm I.D. SS-316 tubing. Two 1000 ml SS 304 cylinder were used as receiver tanks for collecting the liquid products. Corn stover was treated using aqueous ammonia in the first stage and dried in the air as described above. After drying, 10 g of dry ammonia-treated stover was packed into the flow-through reactor. The oven was preheated for 15 minutes, and 2.1 MPa of N2 backpressure was applied to the reactor system before reactor startup. Water was pumped by HPLC pump to the reactor in the second stage. At the completion of the run, the reactor was flushed with water to remove the residual sugar in the treated biomass. The wet solids obtained from the reactor were separated into three portions: One was dried for measurement of weight loss and subjected to composition analysis, while the others were subjected to the enzymatic digestibility test and fermentation tests.

2.3. Enzymatic digestibility test

The enzymatic digestibilities of solid samples obtained from two-stage fractionation were determined in duplicate according to the NREL Chemical Analysis and Testing Standard Procedure [32]. The reaction conditions used for two-stage treated solid sample preparation were as follows: (1) S:L = 1:10 using 15% NH4OH solution (WNH3 =15%), at 60 °C for 24 h in the first-stage treatment using batch reactor and (2) 10 min, 160-220 °C with the flow rates of 8- 22 ml/min of hot water in the second stage. In addition, xylooligomer hydrolysates were prepared under the following conditions: (1) S:L = 1:10 using 15% NH4OH solution (WNH3 =15%) at 60 °C for 24 h in the first-stage treatment using batch reactor and (2) 210 °C, 20 ml/min, and 10 min using percolation reactor. The conditions of the enzymatic digestibility test were pH 4.8 (0.05 mol/l sodium citrate buffer) on a shaker bath agitated at 2.5 Hz at 50 °C. Two different enzymatic digestibility tests were conducted with solid residue after two-stage fractionation and xylooligomer hydrolysate which was collected in the second stage. For the enzymatic digestibility tests of the fractionated solid residue, 15 FPU of GC- 220 per g of glucan supplemented and 30 CBU of β-glucosidase (Novozyme 188) per gglucan were loaded. The initial glucan concentration was 1%

of total liquid and solid. The solid residue samples used in the digestibility tests were wet samples as collected after twostage fractionation. Avicel was put through the same procedure as a reference. For the enzymatic digestibility tests of xylooligomer hydrolysates, 8,000 GXU of Multifect-Xylanase were loaded. The initial xylan concentration was 1% of total liquid.

3. Results and discussion

3.1. RSM

The optimal reaction conditions of the first stage treatment using 15% NH4OH solution (WNH3 =15%) were chosen on the basis of the previous study as follows: 1:10 solid:liquid ratio using a 15% NH4OH solution (WNH3 =15%) and 60 °C for 24 h [23]. In this condition, the maximum lignin recovery was obtained, which was approximately 62% based on lignin in the oven dry untreated corn stover. To simplify the experiment in this study, two variables such as reaction temperature and flow rate in the second stage only were identified as the most significant variables with a range of 160-220 °C, 8-22 ml/min, respectively. A CCD with the Design-Expert (Stat-Ease, Inc., Minneapolis, USA) software was employed to investigate the simultaneous effect of reaction temperature and flow rate of hot water treatment on xylan recovery yield and purity. The performances of various combinations of fractionation conditions are summarized in Table 2. The polynomial equation, describing the xylan recovery yield (*Y1*) as a simultaneous function of reaction temperature and flow rate of hot-water treatment, is shown in Eq. (1)

$$Y_1 = 45.45 + 3.77\,A + 8.63\,B - 5.58\,B^2$$

In addition to the data in Table 2, the detail compositional changes of treated solids and liquids in two-stage fractionation and enzymatic digestibilities of treated solids are summarized in Table 3. One negative phenomenon was observed in the 2nd stage treatment; as reaction temperature increased in the second stage, more xylan was solubilized into liquid (lower part of Table 3); however, the accountability of xylan (xylan content in the solid plus that in liquid) above 200 °C was 70-80% for at all tested flow rates, indicating substantial amount of xylan was decomposed under those conditions. On the other hand, the accountability of glucan was nearly 100% and the glucan content was well preserved.

Table 2
Experimental design and results of the central composite design

Run	Variables		Response	
	(A) Flow rate [ml/min]	(B) Reaction temp. [°C]	Y_1 xylan recovery [%]	Y_2 xylan purity [%]
1	-1	-1	27.6	80.6
2	-1	1	48.1	78.8
3	1	-1	34.8	83.9
4	1	1	50.7	79.2
5	0	0	50.4	82.1
6	0	0	42.7	80.9
7	0	0	50.1	82.3
8	0	0	41.6	79.8
9	-1.41	0	36.3	81.3
10	0	-1.41	22.3	82
11	0	1.41	76.6	45.4
12	1.41	0	50.7	79.2

Table 3

Effects of reaction conditions on the composition of liquid hydrolysates and residual solids in two-stage fractionation [a]
Single-stage treatment (after 1st stage – SAA)

Reaction Conditions		Liquid			Solids			Enzymatic Digestibility [b]	
Time [hour]	Temperature [°C]	Glucan [wt.%]	Xylan [wt.%]	Lignin [wt.%]	Glucan [wt.%]	Xylan [wt.%]	Lignin [wt.%]	Glucan [%]	Xylan [%]
Untreated		-	-	-	34.2	22.3	12.2	19.0	12.5
24	60	0.5	3.6	7.5	33.7	18.7	4.7	-	-

Two-stage treatment (1st stage-SAA and 2nd stage –hot water)

Temperature	Liquid			Solids			Total (liquid + solid)			Enzymatic Digestibility [b]	
	Glucan	Xylan	Lignin	Glucan	Xylan	Lignin	Glucan	Xylan	Lignin	Glucan	Xylan
[°C]	[wt.%]	[wt.%]	[wt.%]	[wt.%]	[wt.%]	[wt.%]	[wt.%]	[wt.%]	[wt.%]	[%]	[%]
SAA-treated solid	-	-	-	33.7	18.7	4.7	33.7	18.7	4.7	-	-
8 ml/min											
190	1.2	8.1	0.7	33.8	8.8	2.3	35.0	16.9	3.0	92.7	77.7
10 ml/min											
170	0.9	6.1	0.6	34.2	13.0	3.4	35.1	19.1	4.0	85.8	70.1
210	1.9	10.7	1.0	33.0	4.4	2.5	34.9	15.1	3.5	96.3	97.4
15 ml/min											
160	0.6	5.0	0.5	34.9	13.1	2.6	35.5	18.1	3.1	81.3	66.7
190	1.4	10.3	0.9	32.9	6.8	2.0	34.3	17.1	2.9	91.9	83.6
220	1.9	10.0	1.2	32.0	3.0	1.6	33.9	13.0	2.8	91.6	100
20 ml/min											
170	0.8	7.8	0.7	33.6	10.9	3.3	34.4	18.7	4.0	88.2	74.9
210	1.8	11.3	1.2	32.2	3.4	0.8	34.0	14.7	2.0	98.3	100
22 ml/min											
190	1.8	11.3	1.2	32.3	5.8	1.6	34.1	17.1	2.8	93.3	86.4

[a] All sugar and lignin content based on the oven-dry untreated biomass. Values are expressed as mean.

[b] Enzymatic digestibilities with the residual solids after two-stage fractionation.

Table 4

ANOVA Analysis for Responses Y_1 [xylan recovery (%)] and Y_2 [xylan purity (%)]

Source	Sum of squares	Degree of freedom	Mean squares	*F-values*	*Prob* $a_{>F}$
For Y_1					
Model	917.62	3	305.87	24.91	0.0002
A	113.74	1	113.74	9.26	0.0160
B	596.30	1	596.30	48.57	0.0001
B^2	207.58	1	207.58	16.91	0.0034
Residual	98.22	8	12.28		
Lack of Fit	31.96	5	6.39	0.29	0.8923
Pure Error	66.26	3	22.09		
For Y_2					
Model	24.98	1	24.98	14.38	0.0035
B	24.98	1	24.98	14.38	0.0035
Residual	17.37	10	1.74		
Lack of Fit	13.32	7	1.90	1.41	0.4234
Pure Error	4.05	3	1.35		

[a] Probability values (*P*-values).

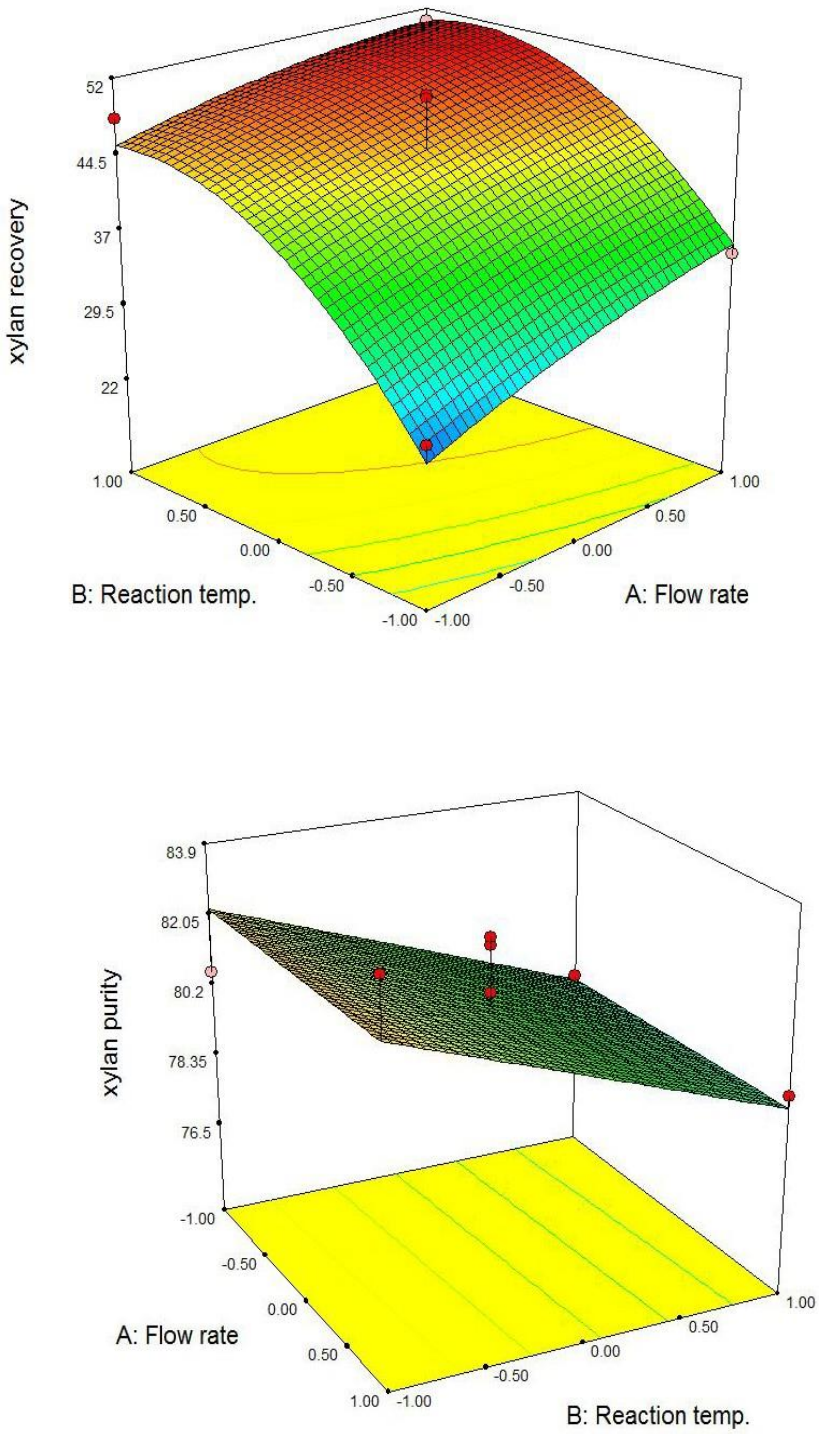

Fig. 2 Response surface curve

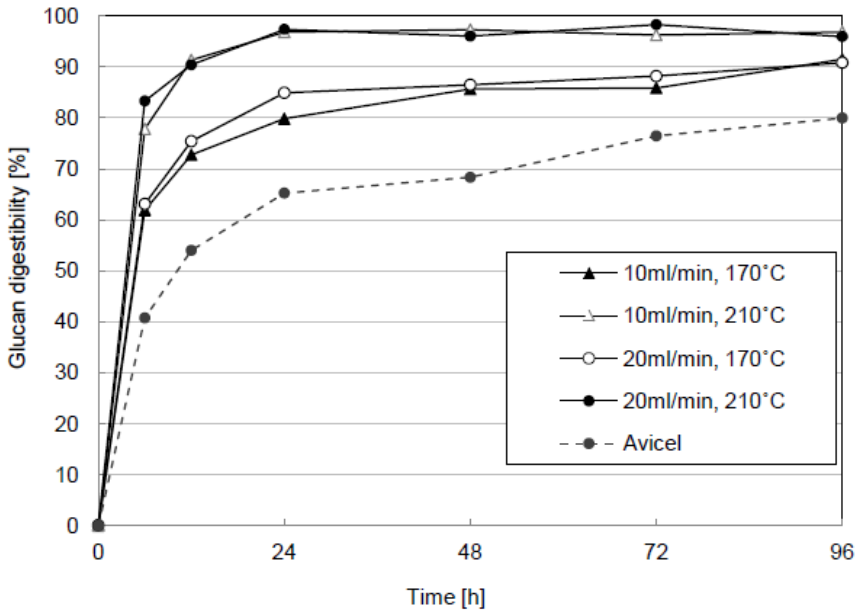

Fig. 3. Enzymatic digestibility of two-stage treated samples

Conclusion

The model equations predicted by RSM indicated that both reaction temperature and flow rate were significant factors for xylan recovery, while only reaction temperature was significant factor for xylan purity in the second stage. The predicted value of xylose recovery yield using RSM was 52%, and the value of purity was 82% under the optimal reaction conditions respectively. From the statistical results, the model equations for xylose recovery yield and purity were significant. Experimental verification of the optimal reaction conditions showed similar values of xylose recovery yield and purity: 55% and 84% respectively.

Cellulase enzymes hydrolyzed two-stage treated solids into fermentable sugars effectively. Accordingly, high yields of ethanol fermentation were obtained using stable *S. cerevisiae* yeast (D5A). Xylooligomer hydrolysate obtained from two-stage fractionation was effectively hydrolyzed by xylanase enzyme. This study demonstrates optimization of a lignocellulosic biomass processing method that allows the production of ethanol from cellulose and the production of other value-added co-products from hemicelluloses sugars and lignin.

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