Energy Fuels **2010,** *24,* 718–727 · DOI:10.1021/ef9009179
Published on Web 12/02/2009

energy&fuels
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Hydrothermal Pretreatment and Enzymatic Hydrolysis of Prairie Cord Grass

Iwona Cybulska,[†] Hanwu Lei,*[,‡] and James Julson[†]

[†]*Department of Agricultural and Biosystems Engineering, South Dakota State University, Brookings, South Dakota 57007 and* [‡]*College of Engineering and Architecture, Washington State University, Richland, Washington 99352*

Received August 24, 2009. Revised Manuscript Received November 6, 2009

The focus of this study was examination of the hydrothermal pretreatment method applied to the lignocellulosic substrate for bioethanol production, represented by prairie cord grass, and comparison between different conditions, based on the yield of glucose after enzymatic hydrolysis. The treatment did not involve any chemicals usage. Hydrothermal pretreatment was conducted in the Parr high-pressure reactor, in the presence of water. After the pretreatment, material was analyzed via high-performance liquid chromatography (HPLC) for products and scanning electron microscopy (SEM) for biomass structure changes. Enzymatic hydrolysis was performed to examine the amount of glucose that was released from pretreated materials. Results were compared based on the conversion rates of glucose and other sugars, as well as the generation of byproducts. Final results suggested that the most efficient pretreatment conditions involved high temperatures (210 °C) and relatively short reaction times (10 min), after which the lignocellulose structure seems to be the most available for enzymes actions. The pretreatment conversion rate in this case reached a level of 97%.

1. Introduction

Processing lignocellulosic biomass to obtain various products, especially bioenergy in the form of biofuels, is a challenging goal of the recent years of research. The most difficult and expensive part of the biomass processing is represented by its hydrolysis−decomposition of complex polymers into easily convertible compounds. Hydrolysis of lignocellulose without any pretreatment has a tendency to achieve low efficiencies.[1]

The biomass biochemical conversion process to ethanol is affected by many factors, the most important of those are substrate-related and enzyme-related. The first category factors are the content of lignin and hemicellulose, as well as cellulose crystallinity and degree of polymerization (DP). Also, an important factor is the amount of accessible surface area, which is necessary for effective enzyme actions. Enzyme-related factors include shear (mixing) or thermal deactivation, inhibition by substrate overloading, inhibition by enzyme overloading. Furthermore, accumulation of cellobiose and glucose can act inhibitory on the enzyme performance.[2] These problems may result in reduced reaction rates.

The low conversion efficiency of cellulose hydrolysis can be improved by applying an efficient pretreatment method. The purpose of pretreatment is mainly lignin removal, as well as reduction of the cellulose crystallinity, degree of polymerization, and particle size. The pore volume and, hence, specific surface area can be significantly increased after an efficient pretreatment. The pretreatment characteristics should include the following: low cost, the possibility to be used on an industrial scale, effectiveness in a wide range of lignocellulosic materials, minimum requirements of preparation and handling prior to the process itself, complete recovery of the lignocellulosic components in usable form, and providing a cellulose fraction possible to be enzymatically converted into glucose at high rate.[3,4]

Research approaches have shown the merits of water as a pretreating agent. Pressure cooking of plant materials using hot water was determined to maximize physical changes and minimize the hydrolysis of cellulose and, therefore, sugar degradation products during pretreatment, while making the pretreated cellulose highly reactive for subsequent enzymatic hydrolysis to achieve maximal glucose yield.[4−7] Physical changes by hydrothermal pretreatment that improve enzymatic hydrolysis of cellulose are well-known and include an increase in pore size to enhance enzyme penetration, and an increase in accessible cellulose by decreasing its crystallinity and association with lignin.[8−11]

Usage of water and high temperatures is a promising alternative to utilization of chemicals (e.g., acid or base hydrolyses).[12,13] The hydrothermal pretreatment process itself

*Author to whom correspondence should be addressed. Tel.: 509-372-7628. Fax: 509-372-7690. E-mail: hlei@tricity.wsu.edu.

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is considered as autohydrolysis of lignocellulosic linkages in the presence of hydronium ions generated from water. During the treatment, hydronium ions are generated also from acetic groups released from hemicellulose. Physical disruption of the lignocellulose structure also occurs, because high pressures are involved. This results in decreased crystallinity of the cellulose, as well as a decrease in the DP value.[14]

During any pretreatment, polysaccharides are being decomposed to oligomers and monomers, while some of the monomers (hexoses pyranosidic structures and pentoses furanosidic structures) are converted to hydroxymethylfurfural (HMF) and furfural. These compounds are considered to be inhibitors for the fermentation and, therefore, should be removed. The lignin fraction is also being depolymerized in water and converted to phenolic groups; however, a repolymerization process also occurs. Repolymerized lignin precipitates on cellulose; it is generally called pseudolignin or Klason lignin.[15] Lignin extraction can be further performed with organic solvents, except for Klason lignin, which binds to cellulose fibers irreversibly. Besides the compounds previously mentioned, several other byproducts are formed during the pretreatment. These include acetic acid (formed during breaking off the acetic groups from hemicellulose), furfural (generated during furfural degradation), formic and levulinic acids (generated during HMF degradation). Some of the phenolic derivatives also are formed from lignin disruption, e.g., vanilin.[16] Hemicellulose recovery is a challenge, because detoxification of this fraction is a difficult task. Detoxification can include the following: steam stripping or evaporation at low pH, which removes acetic acid and furans derivatives; overliming, which removes both furans and phenols; and ion exchange or extraction.[17]

The hydrothermal pretreatment process is fairly easy to perform; there is a relatively low usage of energy (depending on the harshness of the conditions), the process is without the difficult steps of handling and recovery of chemicals (e.g., sulfuric or hydrochloric acid), and equipment corrosion can be excluded here. The process is already applied to lignocellulosic biomass such as wheat straw in European research projects.[18]

Several lignocellulosic biomasses have already been examined as potential feedstock for ethanol production.[1,16,19−21] In this study, prairie cord grass (PCG) was examined as a representative of the herbaceous energy crops. Its distribution is very wide, especially in southwestern and southeastern parts of the United States, as well as in South Dakota and Canada. PCG is a perennial grass, starting its growth in the early spring. It can reach heights up to 3 m, with leaves reaching lengths of 80 cm. Because of its coarseness, PCG is rarely used as animal feed; therefore, using it in ethanol production is a way of utilizing its large amounts that are produced every year. It contains a fair amount of cellulose, which makes it attractive as ethanol feedstock.[22]

Table 1. Prairie Cord Grass (PCG) Composition

component	content [%DM][a]
glucose	33.07 ± 0.37
xylose	13.52 ± 2.00
arabinose	1.59 ± 0.57
lignin	20.96 ± 0.52
ash	5.65 ± 0.04

[a] Expressed in units of percentage dry matter (denoted as %DM).

The main objectives of this study were evaluation of hydrothermal pretreatment efficiency in PCG processing, as well as choosing optimal conditions for the process.

2. Materials and Methods

Prairie cord grass (PCG) was harvested in Brookings, SD. Compositional analysis of the PCG was performed via acid hydrolysis, according to ref 23, and the results are given in Table 1.

2.1. Hydrothermal Pretreatment. Prior to the experiment, PCG was ground to pass through a 1-mm screen (Thomas−Wiley Laboratory Mill, Model 3375-E15, Thomas Scientific, USA). Deionized (DI) water and 8% (w/w) dry matter (DM) of biomass were placed in the jacket-heated Parr reactor (Model HP/HT Pressure Reactor 4570, Parr Instrument Company, Moline, IL), with constant agitation and control of the temperature and pressure. Based on preliminary trials, particle size and DM load were observed to be not significant on the sugar conversion yield. Therefore, particle size and solid concentration were chosen as factors to ensure convenient handling of the material. After preheating to the desired temperature (∼40 min), the reaction time was recorded and mixture was cooled with cooling water using a refrigerated water bath (Type 001-4637/193, Haake, Germany) for ∼1−2 h to achieve room temperature. Certain losses of overall mass occurred during the process, mainly because of material transfers. Decreased mass of the solids fraction was a result of part of the cellulose, hemicellulose, and lignin being removed by dissolution in water. The total overall weight loss during the process was 2%−5%.

The pulp was separated from liquid fraction by vacuum filtration. The pH value of liquid fraction after the process was between 3.51 (after treatment at 210 °C and 10 min) and 4.67 (after treatment at 161.72 °C and 15 min). The filtration cake was washed with ∼300 mL of DI water, filtered again, and stored in the freezer. The liquid fraction was also kept in a freezer for further analyses. The conditions for the solids and liquid fraction yields, as well as glucose content in the solids fraction, are shown in Table 2.

In some other studies, the hydrothermal pretreatment process was applied with addition of catalyst (e.g., potassium hydroxide or sulfuric acid) to activate the autohydrolysis.[7] However, in this study, no extraneous chemicals were added to the process, which eliminates the need for the subsequent recovery of chemicals.

2.2. Hydrolysis. Hydrolysis was performed according to National Renewable Energy Laboratory (NREL) protocol (LAP 009),[24] using cellulase (Novozymes, NS50013) and

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Table 2. Pretreatment Output Data

experiment	solids fraction [g]	liquid fraction [g]	glucose yield in solid fraction [%]
1	19.40	220.43	41.86
2	13.52	248.90	59.90
3	21.24	247.44	36.72
4	13.29	239.82	61.83
5	19.10	189.93	41.13
6	13.03	252.99	63.26
7	13.46	231.82	59.81
8	13.76	235.71	58.79
9	14.06	243.22	57.57
10	13.73	236.48	57.83
11	13.27	236.39	61.15
12	13.20	241.68	61.43

β-glucosidase (Novozymes, NS50010), added in amounts of 15 FPU/gDM and 60 CBU/gDM, respectively. Biomass was placed in the flasks in amounts that were adjusted to achieve 3 g of DM, along with 0.1 M citric buffer with pH 4.8 (50 mL), and DI water was added to make a total volume of 100 mL.

The hydrolysis was conducted in a 100-mL mixture (at pH 4.8), monitored online by collecting 1.5 mL of sample after 0, 3, 6, 12, 24, 36, 48, and 72 h; the testing was performed in duplicate (except for the blind). The concentrations of sugars, as well as byproducts, were measured using high-performance liquid chromatography (HPLC) instrumentation and samples were prepared according to NREL Procedures LAP 013[25] and LAP 015.[26]

2.3. Conversion Rates. To compare the efficiency of pretreatment, as well as the hydrolysis itself (to assess the availability of cellulose structure for enzymes actions), the cellulose-to-glucose conversion rates were calculated according to the following formulas:

$$\text{hydrolysis conversion (\%)} = \frac{\text{glucose amount after hydrolysis}}{\text{glucose amount in raw material}} \times 100 \quad (1)$$

$$\text{pretreatment conversion (\%)} = \frac{(\text{glucose in solid}) + (\text{glucose in filtrate})}{\text{glucose amount in raw material}} \times 100 \quad (2)$$

Here, the conversion rate represents the ratio of the amount of glucose that can be recovered from the pretreated material to the amount of glucose in the material fed to the process.[1]

2.4. Response Surface Analysis. The pretreatment trials were based on central composite experimental design (CCD). A 2^2-factorial central composite design with four replications at the center point was used, giving 12 experiments overall (see Table 3).

Second-order polynomial equations were developed to describe the relationship between independent variables and response variables, such as the concentrations of glucose, xylose, arabinose, acetic acid, etc. The equations are shown below, using X_1 as the temperature (expressed in units of °C) and X_2 as the time (given in minutes); Y_i represents the response variables. The second-degree polynomials (eq 3) were calculated using the statistical package (SAS Institute, Inc., USA) to estimate the response of the dependent variable.

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{11}{X_1}^2 + b_{21}X_2X_1 + b_{22}{X_2}^2 \quad (3)$$

where Yi is the predicted response, X_1 and X_2 are independent variables, b_0 is an offset term, b_1 and b_2 are linear effects, b_{11} and b_{22} are squared effects, and b_{21} is the interaction term.

Table 3. Experimental Design for Prairie Cord Grass (PCG) Hydrothermal Pretreatment

experiment	factor 1 (temperature) [°C]	factor 2 (time) [min]
1	170.00	10.00
2	210.00	10.00
3	170.00	20.00
4	210.00	20.00
5	161.72	15.00
6	218.28	15.00
7	190.00	7.93
8	190.00	22.07
9	190.00	15.00
10	190.00	15.00
11	190.00	15.00
12	190.00	15.00

2.5. Scanning Electron Microscopy (SEM) Analysis. The difference in the lignocellulosic structure of PCG before and after the hydrothermal pretreatment was measured by scanning electron microscopy (SEM) (Hitachi Model 3500). The SEM images show how the raw structure can be opened during the treatment, which enhanced the surface area available for the enzyme actions. Pictures were taken at 30.0 kV and magnifications between 350× and 2300×.

3. Results and Discussion

3.1. Hydrothermal Pretreatment. As shown in Figures 1 and 2, the production of sugars varied with the conditions of the process. The most efficient glucose production from the lignocellulosic structure during the hydrolysis was obtained in the case of the material pretreated at high temperature (210 °C) and short reaction time (10 min), represented by experiment 2. The lowest process efficiency was observed in the case of applying relatively low temperature (170 °C) and short reaction time (10 min). The comparison of glucose production during online monitoring of the hydrolysis among all 12 experiments can be seen in Figure 1.

Comparison among four different conditions of the hydrothermal pretreatment, in terms of sugars and byproduct generation during online-monitored hydrolysis, can be found in Figures 2 and 3. As shown in Figure 3, byproduct generation was not significant during the hydrolysis. This was a result of a thorough washing of the cellulose fraction after the treatment application. Also, a lack of significant lactic acid production proved that no bacteria infection occurred during the process. Acetic acid production was observed to be highest in the case of either low temperature or short reaction time, and lowest in the case of high-temperature application. Acetic acid was produced by the decomposition of hemicellulose during enzymatic (or chemical) hydrolysis. The generation of acetic acid can be avoided by effective transfer of hemicellulose to the liquid fraction during pretreatment. It can be seen that, in the case of high-temperature application, hemicellulose was removed most effectively, resulting in low acetic acid and xylose production during the hydrolysis. However, most of the xylose was converted to furfural during the pretreatment, resulting in a high concentration of this inhibitor in the liquid fraction.

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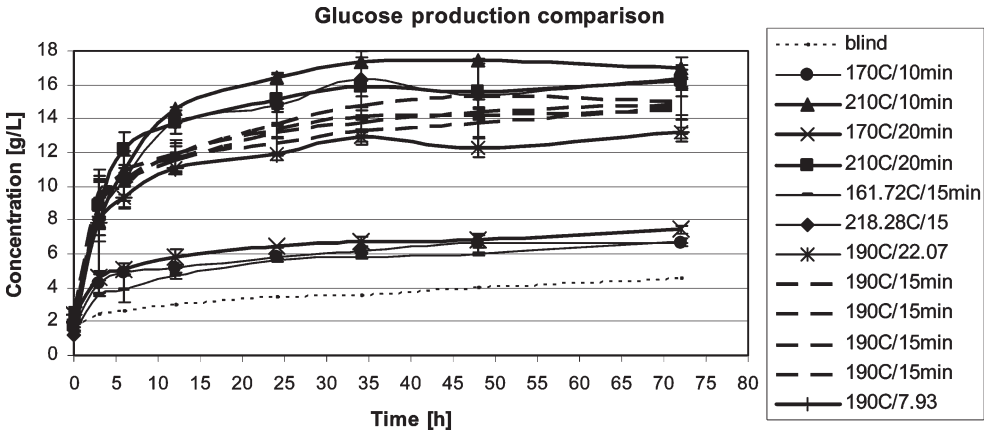

Figure 1. Glucose production comparison among different processing conditions (temperatures of 161.72−218.28 °C and reaction times of 7.93−22.07 min).

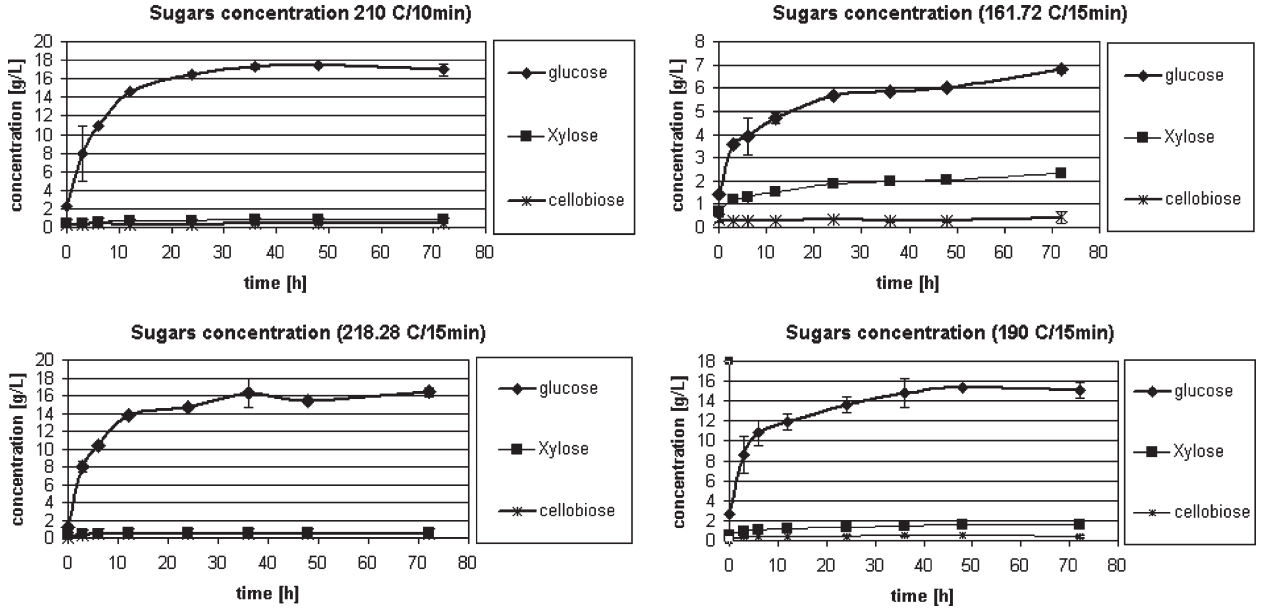

Figure 2. Sugars produced during the online-monitored hydrolysis for Experiment 2 (210 °C/10 min), Experiment 5 (161.72 °C/15 min), Experiment 6 (218 °C/15 min), and Experiment 7 (190 °C/15 min).

Table 4. Concentration of Sugars in the Filtrate after Hydrothermal Pretreatment

	Concentration [g/L]			
experiment	glucose	xylose	arabinose	cellobiose
1	2.84	3.33	1.10	0.53
2	1.34	1.54	0.50	0.53
3	2.56	2.99	1.28	0.52
4	0.94	0.62	1.34	0.38
5	3.16	3.68	0.99	0.52
6	0.84	1.14	1.37	0.37
7	1.65	2.80	1.28	0.41
8	1.51	4.84	1.04	1.99
9	1.46	3.89	1.09	0.43
10	1.49	3.92	1.05	1.40
11	1.45	3.99	0.81	2.30
12	1.45	3.94	1.05	0.44

Table 5. Concentration of Byproducts in the Filtrate after Hydrothermal Pretreatment

	Concentration [g/L]				
experiment	acetic acid	lactic acid	furfural	HMF	xylitol
1	1.08	0.48	0.28	0.17	0.24
2	4.04	1.48	4.11	1.29	1.76
3	1.36	0.54	0.23	0.34	0.34
4	4.08	1.56	3.60	1.40	0.41
5	0.93	0.41	0.23	0.15	0.21
6	4.28	1.53	2.29	1.36	0.40
7	2.11	0.81	0.67	0.52	0.65
8	3.22	0.97	1.70	0.82	0.05
9	2.72	0.99	1.71	0.70	0.75
10	2.63	0.97	1.11	0.62	0.95
11	2.72	0.83	0.95	0.71	1.24
12	2.47	0.92	1.72	0.41	0.67

As mentioned previously, hemicellulose and the products of its degradation were removed to the filtrate after hydrothermal pretreatment. The filtrate was also analyzed for the presence of sugars and inhibitors (without any post-treatment). The results are shown in Tables 4 and 5. To be able to use hemicellulose sugars in the hydrolysis and further in the fermentation process, liquid fraction must be detoxicated, which is a labor-consuming and expensive procedure. Moreover, the sugars that are present in the filtrate are mostly pentoses, which do not have a feasible application in fermentation process currently. Instead, C-5 sugars can be utilized in cattle feed production.[18]

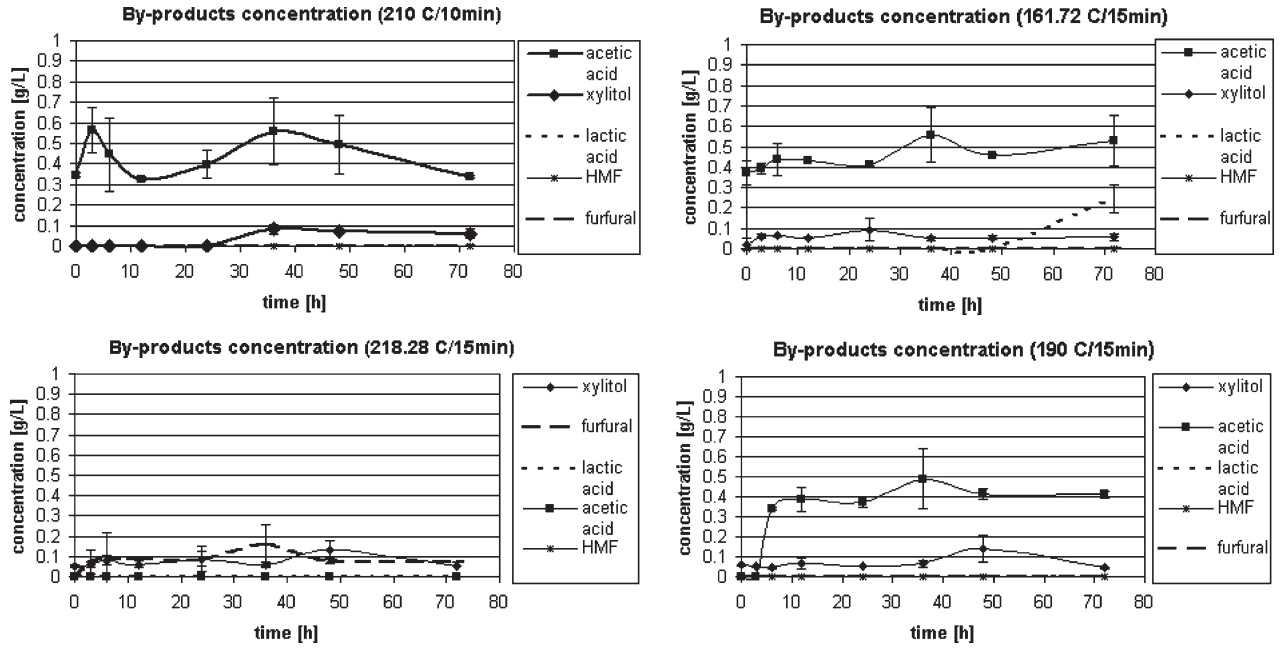

Figure 3. Production of byproducts during the online-monitored hydrolysis for Experiment 2 (210 °C/10 min), Experiment 5 (161.72 °C/15 min), Experiment 6 (218 °C/15 min), and Experiment 7 (190 °C/15 min).

Table 6. Conversion Rates of Glucose during Hydrolysis and Hydrothermal Pretreatment

	Conversion Rate [%]	
experiment	hydrolysis	pretreatment
blind	45.66	
1	53.17	72.79
2	94.53	97.96
3	67.57	84.24
4	87.49	90.09
5	54.97	74.86
6	86.39	90.82
7	73.45	78.90
8	91.85	96.19
9	87.01	91.45
10	84.36	87.36
11	80.80	85.12
12	78.75	83.02

3.2. Conversion Rates. The conversion rates for each condition of the pretreatment are shown in Table 6. The highest conversion rates, for hydrolysis (94.53%) as well as pretreatment (97.96%), were assigned to Experiment 2, representing the following conditions: 210 °C and 10 min. In cases of higher temperature (218 °C) and longer time (15 min) (represented by Experiment 6), an ~8% decrease in the rate of glucose conversion was observed. Lower temperatures (represented by Experiments 1, 3, and 5) gave much lower rates of glucose conversion (< 70%). However, it can be seen that even cooking at relatively low temperature (Experiment 5, 161.72 °C) gave a conversion rate of the hydrolysis that was higher than that of the nontreated sample (blind). To illustrate changes in the composition of biomass during hydrothermal pretreatment, material balances for the most-effective and least-effective conditions were performed (see Table 7).

3.3. SEM Analysis. A comparison of the raw sample with samples that have been pretreated under four different conditions can be found in Figures 4 and 5.

Table 7. Example Material Balance

			Output			
	Input		Liquid		Solid	
component	[%]	[g]	[%]	[g]	[%]	[g]
Experiment Conditions: 210 °C/10 min						
glucose	33.00	8.43	1.30	0.33	31.00	8.10
hemicellulose	15.60	3.99	5.90	1.52	9.70	2.47
lignin	21.00	5.38	18.50	4.74	2.50	0.64
ash	5.65	1.54	0.00	0.00	5.65	1.54
Experimental Conditions: 161.72 °C/15 min						
glucose	33.00	8.46	2.30	0.60	31.00	7.86
hemicellulose	15.60	4.00	4.00	0.93	11.57	3.07
lignin	21.00	5.38	0.00	0.00	21	5.35
ash	5.65	1.54	0.00	0.00	5.65	1.54

As shown in the photographs, raw PCG had a different fiber structure. The pores did not occur in large amounts, and the entire structure was more closed. With regard to the pretreated samples, it can be seen that, in each of the examples, the fiber structure was highly porous. Pores sizes were similar in Experiments 5, 6, and 7 (see Figure 4); however, in the case of Experiment 2 (210 °C/10 min), pore sizes were much smaller (practically 10 times smaller). Microstructure analysis results can be found in Table 8. These conditions gave the highest glucose yields, which was surely enhanced by the effect of "spongy" structure caused by multiple small pores opened during the pretreatment. The largest pores sizes were measured in samples that had been pretreated at 190 °C for 15 min. This also resulted in high enzymatic conversion (~90%).

3.4. Response Surface Analysis. Twelve experiments were performed using different levels of the two most important factors affecting the hydrothermal treatment: temperature and reaction time. The levels were chosen according to CCD. Second-order polynomial equations were developed to describe the relationship between independent variables and four response variables (the concentration of glucose, xylose,

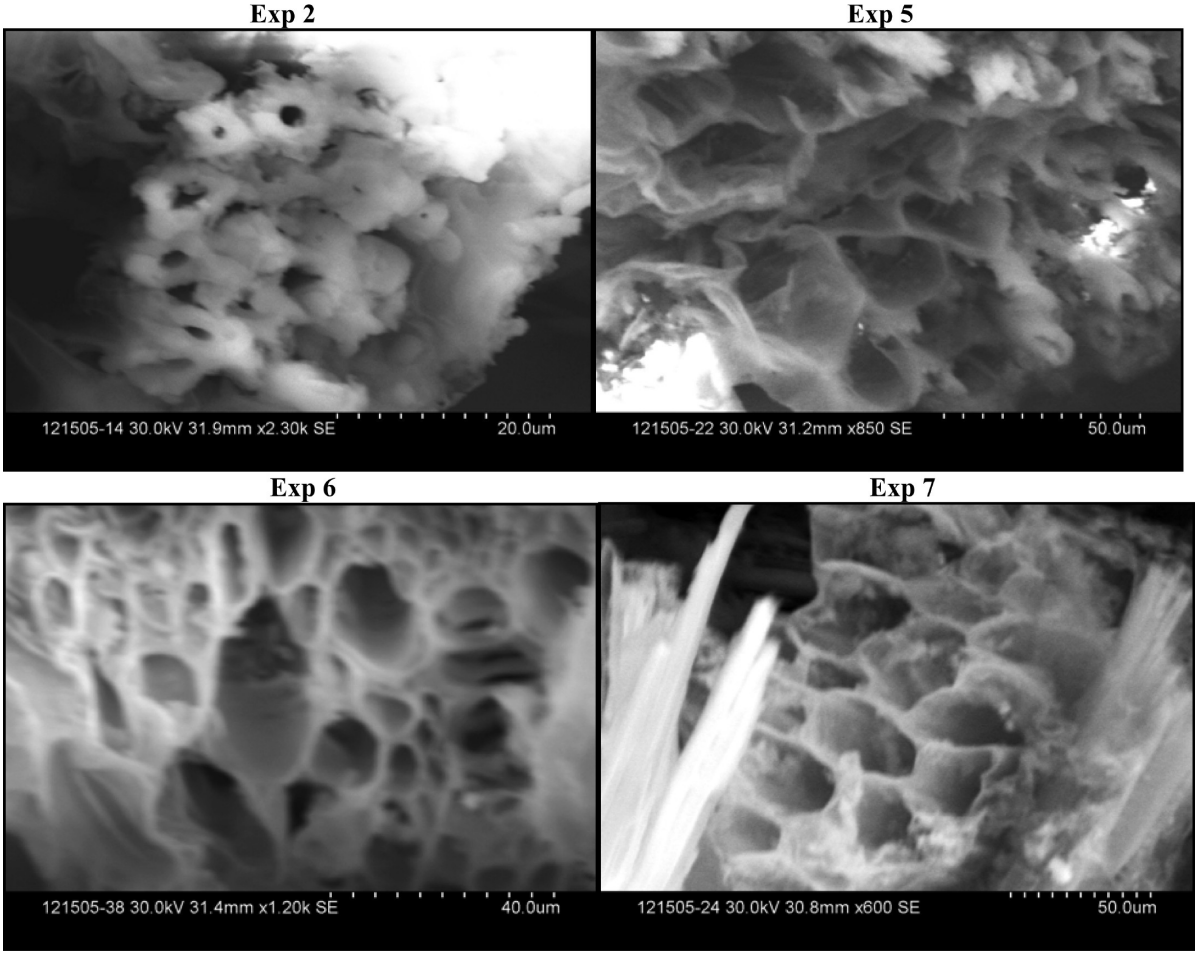

Figure 4. Scanning electron microscopy (SEM) photographs of samples pretreated under various conditions: 210 °C/10 min (Experiment 2), 161.72 °C/15 min (Experiment 5), 218 °C/15 min (Experiment 6), and 190 °C/15 min (Experiment 7).

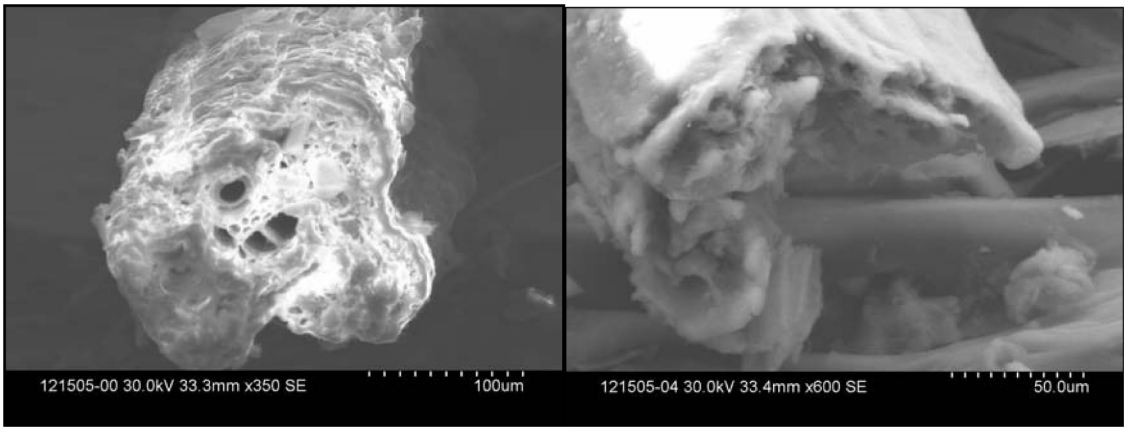

Figure 5. SEM photographs of raw prairie cord grass (PCG).

Table 8. SEM Analysis Results

Experimental Conditions		Pore Data	
temperature [°C]	time [min]	average length [μm]	average width [μm]
210	10	2.24	1.81
161.72	15	9.56	6.79
218	15	17.1	10.5
190	15	32.6	17.1

Table 9. ANOVA Table for Glucose Concentration

SOV	DF	SS	MS	*F*-value	*P*-value	% contrib
temperature	1	267.2	267.2	352.1**	<0.001	95.1
time	1	4.566	4.566	6.016*	0.030	1.6
error	12	9.107	0.7589			
total	14	280.9				

arabinose, and acetic acid). The equations can be found below, with X_1 as the temperature (°C), X_2 the time (in minutes), Y_1 the concentration of glucose ($R^2 = 0.92$), Y_2 the concentration of xylose ($R^2 = 0.84$), Y_3 the concentration of cellobiose ($R^2 = 0.80$), and Y_4 the concentration of

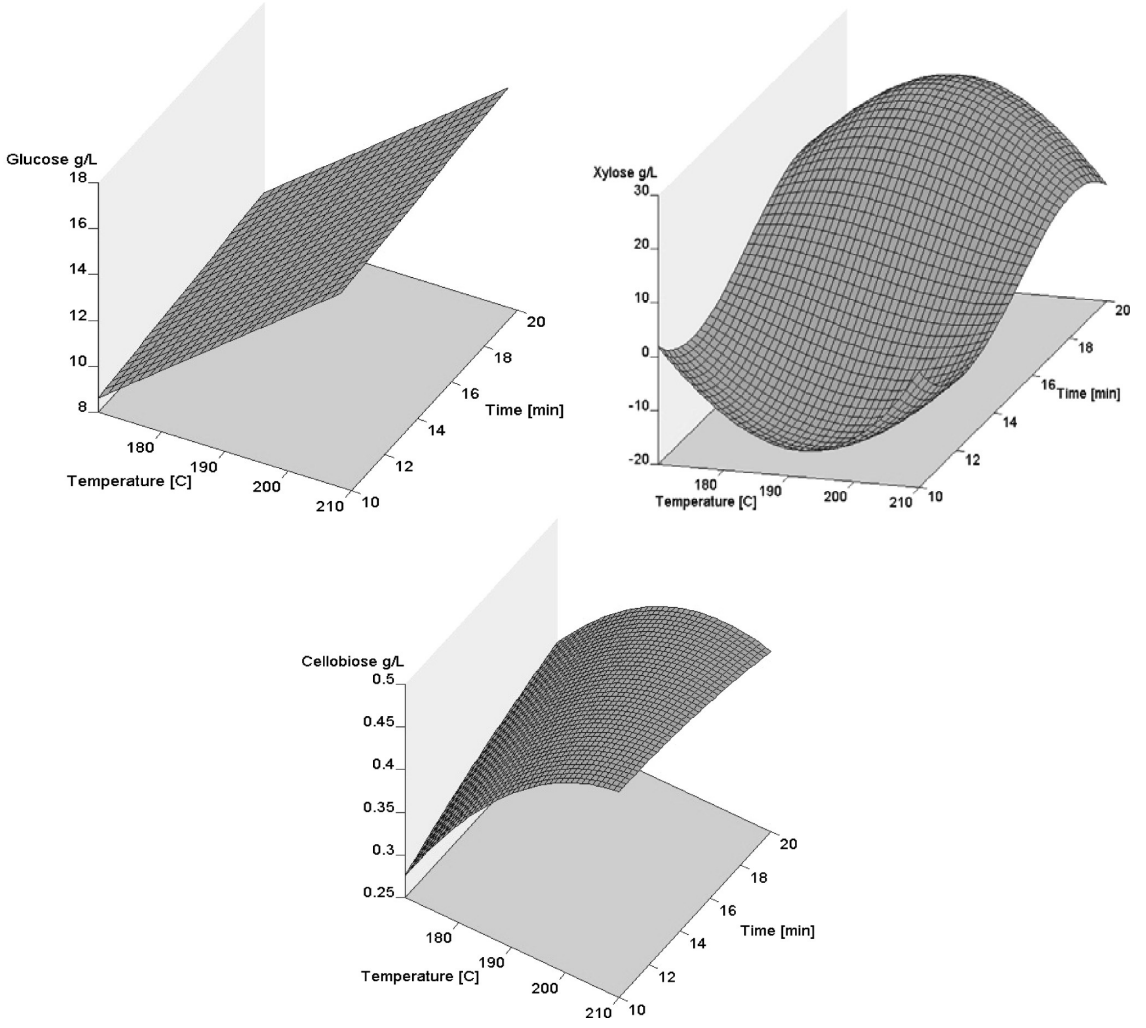

Figure 6. Response surfaces for glucose, xylose, and cellobiose (hydrolyzed samples).

acetic acid ($R^2 = 0.89$).

$$Y_1 = 212 + 2.07X_1 + 1.26X_2 + 0.00382X_1X_2 - 0.00476{X_1}^2 - 0.0143{X_2}^2 \quad (4)$$

$$Y_2 = 11.3 - 0.0661X_1 + 0.09X_2 - 0.00227X_1X_2 + 0.00015{X_1}^2 + 0.0108{X_2}^2 \quad (5)$$

$$Y_3 = 5.35 - 0.0396X_1 + 0.261X_2 - 0.000758X_1X_2 + 0.000068{X_1}^2 + 0.0045{X_2}^2 \quad (6)$$

$$Y_4 = 5.37 - 0.0702X_1 + 0.0344X_2 - 0.000148X_1X_2 + 0.000198{X_1}^2 + 0.0018{X_2}^2 \quad (7)$$

The R^2 values showed that the models for each response variable explain the process relationships well. Also, the corresponding ANOVA table for glucose can be seen in Table 9, showing that the variation was strongly dependent on temperature (which explains the variation of $>90\%$).

The predicted optimal conditions for the hydrothermal pretreatment were developed based on the regression equation (response surfaces plots are shown in Figures 6 and 7). The maximum glucose production (16.99 g/L) was achieved under the following conditions: a temperature of 210 °C and a reaction time of 10 min.

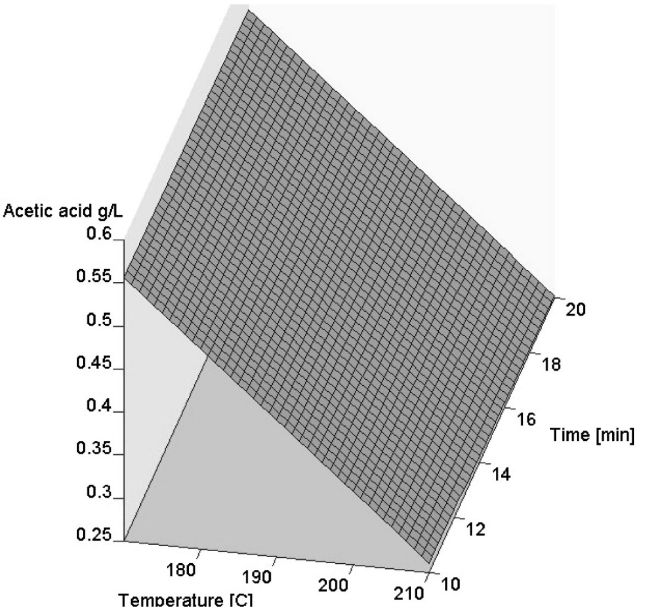

Figure 7. Response surface for acetic acid (hydrolyzed samples).

As shown in Figure 6, the factor of time had a minor effect on the glucose generation, when compared to temperature,

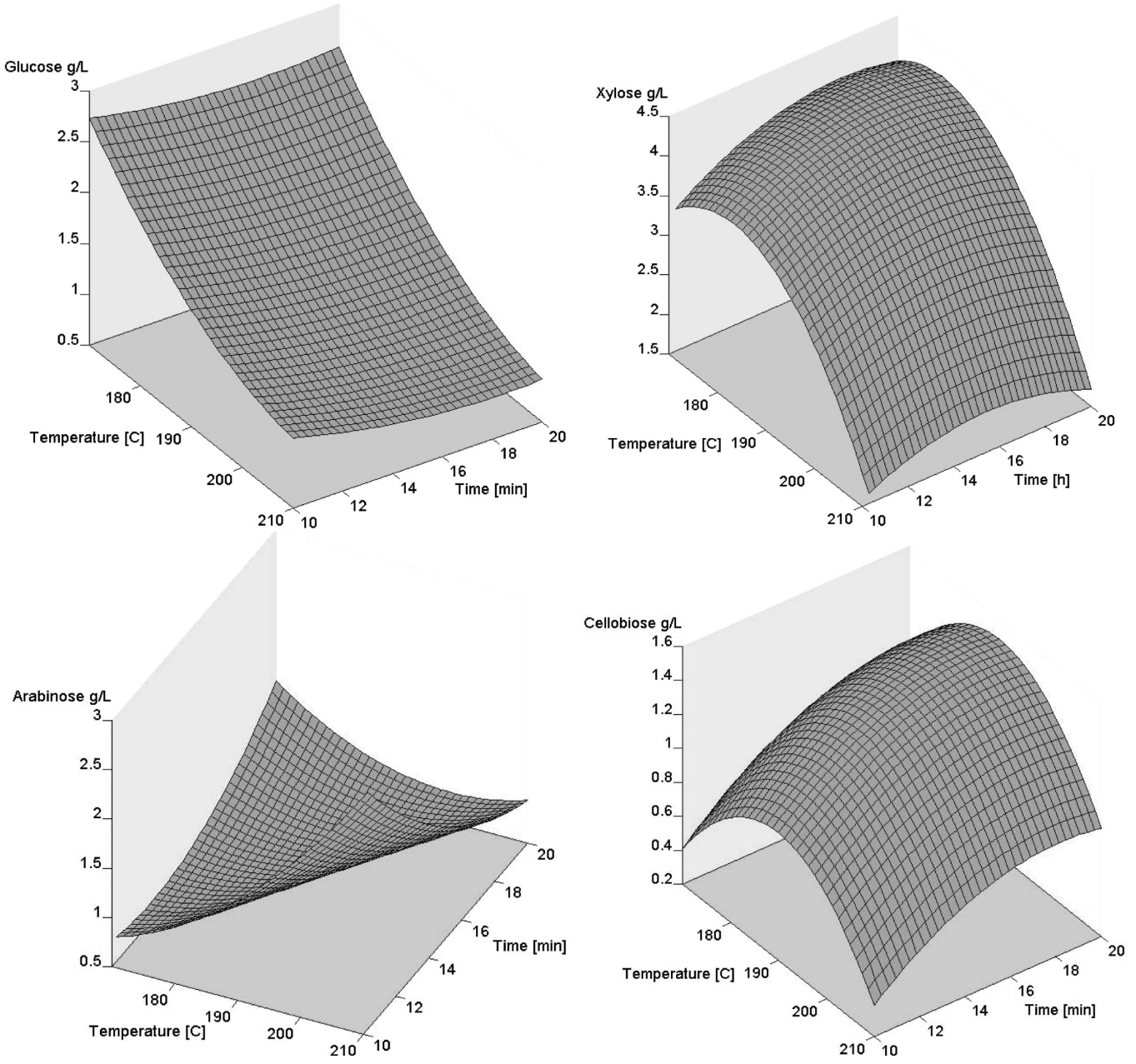

Figure 8. Response surface for glucose, xylose, arabinose, and cellobiose (filtrate).

which had a major effect on glucose generation (glucose concentration increases with temperature). Xylose production was dependent on both time and temperature, and cellobiose production was dependent more on temperature than time. Acetic acid production was not dependent on any change in time; however, temperature has great influence on the acetic acid production (the increase in temperature causes a decrease in acetic acid production), which can be seen in Figure 7.

The filtrate was also analyzed following CCD models, and the regression equations are presented in eqs 8−12: glucose (Y_5, $R^2 = 0.99$), xylose (Y_6, $R^2 = 0.78$), arabinose (Y_7, $R^2 = 0.69$), cellobiose (Y_8, $R^2 = 0.68$), acetic acid (Y_9, $R^2 = 0.98$). In addition, analysis was made for the inhibitors (byproduct) present in the filtrate, which were lactic acid (Y_{10}, $R^2 = 0.97$), HMF (Y_{11}, $R^2 = 0.95$), and furfural (Y_{12}, $R^2 = 0.74$).

$$Y_5 = 36.6 - 0.322X_1 - 0.0742X_2 - 0.00309X_1X_2 + 0.000754{X_1}^2 + 0.0037{X_2}^2 \quad (8)$$

$$Y_6 = 90.5 + 0.982X_1 + 0.743X_2 - 0.00143X_1X_2 - 0.00265{X_1}^2 - 0.0143{X_2}^2 \quad (9)$$

$$Y_7 = 12 - 0.2X_1 + 0.842X_2 - 0.00689X_1X_2 + 0.000854{X_1}^2 + 0.0133{X_2}^2 \quad (10)$$

$$Y_8 = 1.86 - 0.0168X_1 + 0.0541X_2 - 0.000335X_1X_2 + 0.000052{X_1}^2 + 0.00019{X_2}^2 \quad (11)$$

$$Y_9 = 13.4 + 0.0884X_1 + 0.141X_2 - 0.000602X_1X_2 - 0.000038{X_1}^2 + 0.0068{X_2}^2 \quad (12)$$

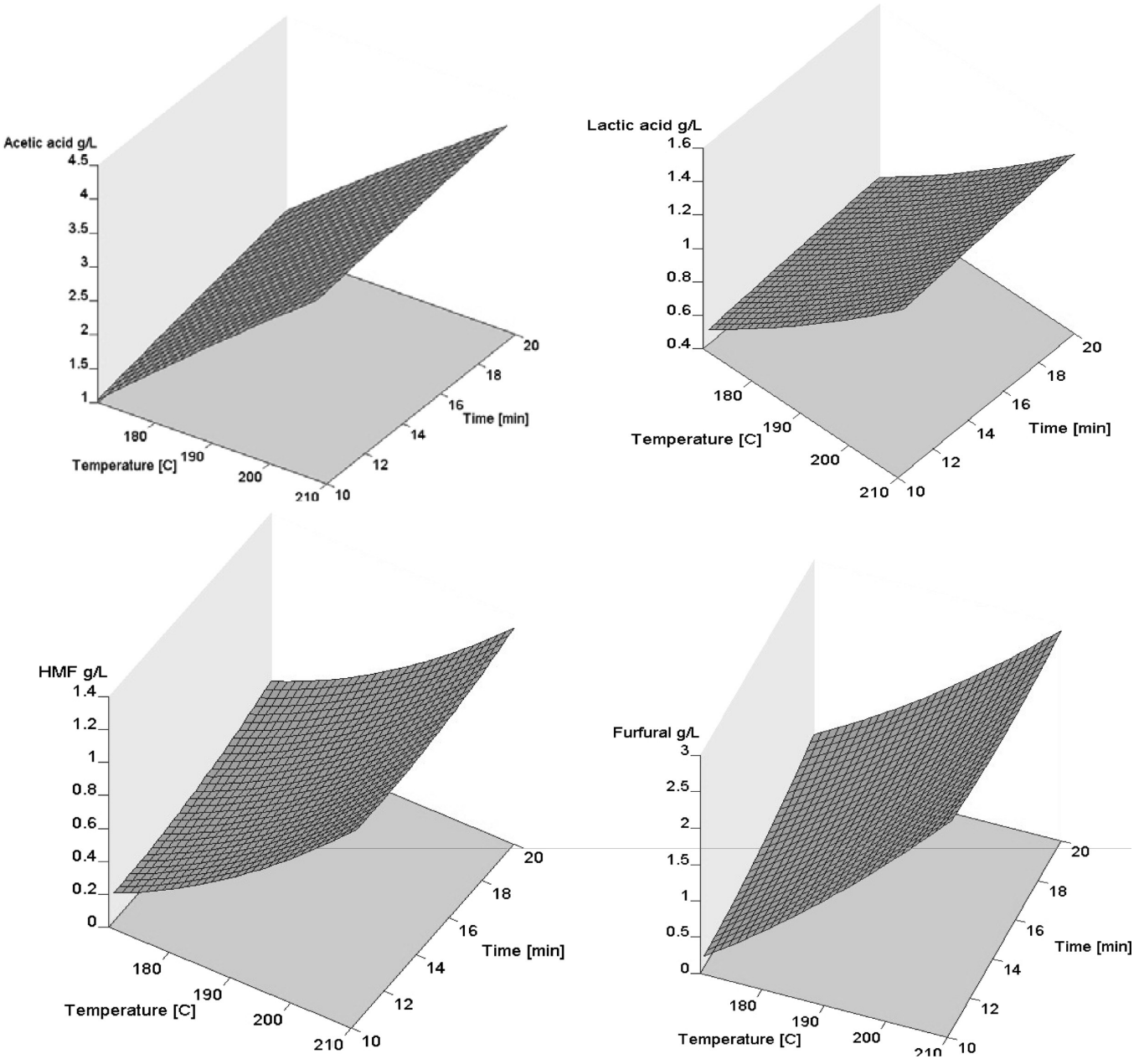

Figure 9. Response surfaces for the byproducts: acetic acid, lactic acid, HMF, and furfural (filtrate).

$$Y_{10} = 0.537 - 0.0187X_1 - 0.00432X_2 + 0.0000469X_1X_2 + 0.000107{X_1}^2 + 0.00014{X_2}^2 \quad (13)$$

$$Y_{11} = 4.28 - 0.0631X_1 - 0.0145X_2 - 0.000158X_1X_2 + 0.000236{X_1}^2 + 0.00206{X_2}^2 \quad (14)$$

$$Y_{12} = 0.479 - 0.0634X_1 + 0.0983X_2 - 0.00112X_1X_2 + 0.000378{X_1}^2 + 0.00458{X_2}^2 \quad (15)$$

The response surface plots of sugars and byproduct components in the filtrate (Figures 8 and 9) were developed based on eqs 8−15 with their acceptable R^2 values, suggesting the applicability of the models and explaining the process relationships well, using these prediction models.

In case of the filtrate (Figures 8 and 9), the change in reaction time did not seem to be significant for glucose and xylose production; however, it did influence the release of arabinose and cellobiose. Temperature had a major influence on the production of all sugars. The release of byproducts into the filtrate was strongly dependent on temperature (increasing as the temperature increases), but not on the change in reaction time. Temperature had a significant effect on both the conversion rates of pretreatment and enzymatic hydrolysis, unlike the change in reaction time, which influenced only the pretreatment conversion rate since in this filtrate calculation was taken into account.

The experimental conversion rates have been given in Table 6. The predicted hydrolysis and pretreatment conversion rates have been calculated using eqs 16 and 17, respectively. The correlation coefficient of determination (R^2) is equal to 0.94 in the case of the hydrolysis conversion rate and 0.84 in the case of the pretreatment conversion rate, which implies that the quadratic regression model can be used to explain the conversion reaction. The sample variation of

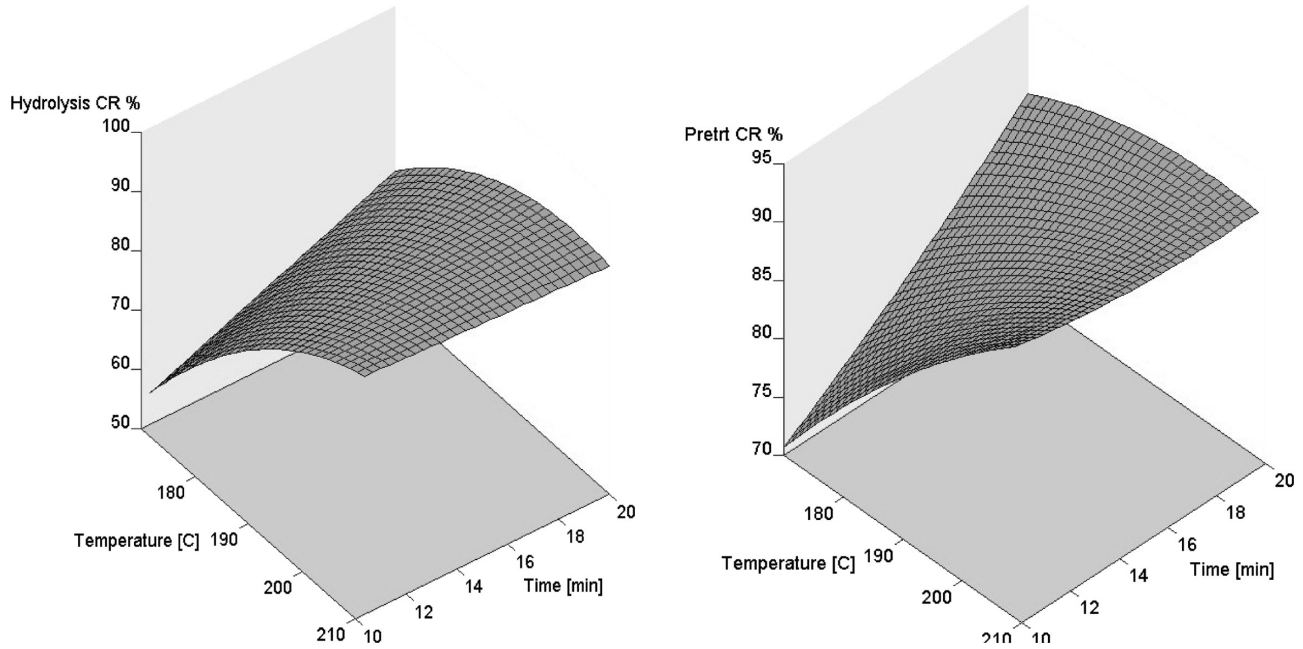

Figure 10. Response surfaces for the glucose conversion rates for hydrolysis and pretreatment.

95.1% for glucose production is attributed to independent variable 1 (temperature) and 1.6% is attributed to independent variable 2 (reaction time).

$$Y_{13} = 725 + 7X_1 + 9.96X_2 - 0.0486X_1X_2 - 0.0148{X_1}^2 + 0.00235{X_2}^2 \quad (16)$$

$$Y_{14} = 270 + 2.66X_1 + 9.04X_2 - 0.0483X_1X_2 - 0.00421{X_1}^2 + 0.0281{X_2}^2 \quad (17)$$

The predicted optimum levels of reaction time and temperature of hydrothermal reaction were obtained by applying the regression analysis to eq 17. Figures 6 and 10 represent surface plots for the hydrothermal conditions of glucose production. The maximum conversion rate of 97.96% appeared at a reaction temperature of 210 °C and a reaction time of 10 min.

4. Conclusion

Hydrothermal pretreatment of lignocellulosic herbaceous materials is a promising method, especially because of its lack of chemical usage and its simplicity. Good results were obtained along with carefully optimized hydrolysis. Based on the results, the most efficient pretreatment conditions were high temperature and short reaction time (210 °C/10 min), giving the highest pretreatment conversion rate (97.96%) and hydrolysis conversion rate (94.53%). Therefore, it is possible to enhance the conversion of untreated material in the hydrolysis by 48.87% with hydrothermal pretreatment and the use of no chemicals. The lowest glucose yields were observed at low temperatures, even with long reaction time. Therefore, it can be concluded that temperature had significant influence on the release of glucose during the hydrolysis, which was also confirmed by the analysis of variance (ANOVA) (see Table 9). Furthermore, the online-monitored hydrolysis results indicate that the duration of the process was shortened to ~36−40 h, instead of 72 h in the case of this material and pretreatment method. The glucose production increase was relatively low (2−10%) during the final stage of hydrolysis (last 40−72 h). Moreover, a thorough washing of the solid fraction yielded a very low amount (or even an absence) of inhibitory byproduct in the hydrolysis mixture. Most of the inhibitors and hemicellulose sugars were found in the filtrate, which also confirms the effectiveness of hydrothermal treatment toward herbaceous materials prior to its hydrolysis and further ethanol fermentation.

Acknowledgment. This work was supported by funding from the South Dakota Research and Commercialization Council.