Bioresource Technology 131 (2013) 101–112

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Optimization of clean fractionation process applied to switchgrass to produce pulp for enzymatic hydrolysis

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HIGHLIGHTS

- Clean fractionation (CF) processing was performed on switchgrass (SG).
- Processing mass balances and composition analyses were performed on the pulp, hemicellulose and lignin separated fractions.
- Pulp fractions were enzymatically hydrolyzed and glucose yields were measured.
- High lignin and xylan removal from biomass were obtained.
- Enzymatic hydrolysis produced high glucose yields (more than 90% were obtained for selected optimal conditions).

ARTICLE INFO

Article history:
Received 22 August 2012
Received in revised form 7 November 2012
Accepted 16 December 2012
Available online 23 December 2012

Keywords:
Organosolv
Clean fractionation
Switchgrass
Enzymatic hydrolysis
Response surface methodology

ABSTRACT

The purpose of this study was to fractionate switchgrass (SG) to obtain hemicellulose-, lignin-rich fractions and highly digestible pulp, using a clean fractionation (CF) approach. The main objective was to produce highest glucose yield in the enzymatic hydrolysis of pulp. Effects of processing factors such as time (10–50 min), temperature (120–160 °C), catalyst concentration (0.21–0.93% w/w sulfuric acid) and organic solvent mixture composition (7–43% w/w methyl isobutyl ketone) were evaluated. Response surface methodology and central composite design were used for process optimization and statistical analyses. High lignin (75–93%) and xylan (83–100%) removal from biomass were obtained, leaving solid pulp rich in glucan (78–94%). High enzymatic hydrolysis glucose yields (more than 90%) were obtained for selected optimal conditions. Pulp can be used for ethanol production while separated xylan and lignin fractions can be used as a feedstock for value-added products which suggests the applicability of clean fractionation technology in a biorefinery concept.

1. Introduction

Lignocellulosic materials have been proven to be abundant and promising sources for bio-based products production, including fuel (e.g., ethanol), polymers and wide variety of chemicals (Alvira et al., 2010; Kamm et al., 2006). However, prior to processing, any lignocellulosic biomass will require an effective pretreatment method, due to its complex structure. Lignocellulose is composed of three main building blocks including cellulose, hemicelluloses and lignin. Cellulose is a crystalline polymer of glucose units linked by β-glycosidic bonds. Hemicellulose is composed of linear and branched polymers of C-5 sugars (xylose and arabinose) and the C-6 sugars (glucose, mannose, and galactose), which are easily hydrolysable. Lignin is an amorphous, heterogeneous polymer which is highly hydrophobic and insoluble during enzymatic hydrolysis. It is mainly built from phenolic compounds derived from coniferyl, sinapyl and coumaryl units. Lignin is connected to carbohydrate components of lignocellulose by α-aryl and β-aryl ether bonds, which can be cleaved in the presence of hydronium ions (Kamm et al., 2006). Lignin can be divided in acid soluble lignin (ASL) and acid insoluble lignin (AIL). ASL is soluble fraction of lignin which is measured by UV–Vis spectroscopy while AIL is the solid residue remaining after a two step hydrolysis measured gravimetrically (Sluiter et al., 2008c).

Cellulose when hydrolyzed to glucose can be converted to ethanol through well established fermentation. However, cellulose is trapped inside the crosslinking structure of the lignocellulose matrix, which limits enzymes access. Hence, pretreatment which removes hemicelluloses and lignin or breaks down the surrounding matrix of lignin and hemicellulose and disrupts the crystalline structure of cellulose prior to the enzymatic hydrolysis is crucial to obtain high conversion of cellulose to glucose during enzymatic

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http://dx.doi.org/10.1016/j.biortech.2012.12.118

hydrolysis (Alvira et al., 2010). Lignin and hemicelluloses, except for acting as a physical barrier, can be a source of the inhibitory compounds such as furan derivatives, weak acids and phenolic compounds, which limit the performance of fermentative microorganisms (Alvira et al., 2010). Lignin can also be responsible for non-productively binding of cellulolytic enzymes (Alvira et al., 2010). Also, reprecipitation of soluble lignin can cause cellulose fibers to become more recalcitrant to enzymatic digestion and thus fermentation (Zhao et al., 2009). Therefore, removal of hemicelluloses and lignin before hydrolysis and fermentation helps to avoid these problems. Pretreatment step contributes to high processing costs, hence the entire lignocellulose polymer should be utilized to generate profit and compensate for the added expenses of the pretreatment. Thus, an efficient pretreatment method should depolymerize and fractionate lignocellulose to produce pure streams of cellulose, hemicellulose and lignin, which can be processed into wide variety of products. The only method that currently provides such opportunity is organosolv treatment, which utilizes the affinity of lignocellulosic components to different solvents.

Organosolv treatment of wood has been employed to produce cellulose pulp in the paper industry since the early 20th Century. Organosolv treatment utilizes different affinities of the lignocellulosic components to various solvents, which leads to fractionation into three streams: cellulose, hemicellulose and lignin. Since the process was found effective in producing enzymatically digestible cellulose, it began to be increasingly investigated as a pretreatment method for bioethanol production (Arato et al., 2005; Goh et al., 2011). Organosolv pretreatment at present is an expensive procedure as compared to other leading pretreatment processes (dilute acid or base pretreatment); however, it can provide some valuable by-products. Separation of the lignocellulose components and utilization of all the output streams and by-products might lead the organosolv pretreatment to be a promising one for biorefining lignocellulosic feedstock in future (Zhao et al., 2009). Organosolv lignin creates an opportunity for generating value-added products (especially chemicals and plastics), since it is free of contaminants and highly phenolic (Brudecki et al., 2012; Cybulska et al., 2012b; Mansouri and Salvadó, 2006).

Many different solvent combinations and various treatment conditions have been examined for the organosolv process. The most common solvent used for lignin extraction is ethanol (Arato et al., 2005; Goh et al., 2011). However, many other organic solvents, such as organic acids (e.g., acetic acid), ketones (e.g., acetone) or esters (e.g., ethyl acetate) have a high affinity towards lignin, which makes them promising as well (Chum et al., 1990; Zhao et al., 2009). Furthermore, solvents immiscible with water improve phase separation after the process, ensuring better fractionation of the lignocellulosic components, extracting lignin to the organic phase and hemicellulose sugars to the aqueous phase. This approach has been used in the clean fractionation process, which was developed by the National Renewable Energy Laboratory (NREL), and then adapted by other researchers (Black et al., 1998; Brudecki et al., 2012; Cybulska et al., 2012a). Catalysts have been proven to enhance the delignification process, resulting in higher lignin yields and improved enzymatic digestibility of cellulose at relatively low (below 180 °C) temperatures (Chum et al., 1990; Cybulska et al., 2012a).

Feedstocks for the organosolv process are usually softwood or herbaceous materials (either agricultural wastes or energy crops), having relatively high lignin content. These include poplar (Black et al., 1998), wheat straw (Sidiras and Koukios, 2004), or prairie cordgrass (Brudecki et al., 2012; Cybulska et al., 2012a). Switchgrass is also a potential feedstock, and has gained popularity as a bioethanol feedstock due to its relatively high enzymatic digestibility, high productivity, low agricultural requirements and relatively low growing costs (Keshwani and Cheng, 2009; Kim et al., 2011; Sanderson et al., 2006). Switchgrass is highly adaptable to various atmospheric conditions, which enabled it to inhabit regions in the entire contiguous North America, especially the Great Plains and the Midwest (Keshwani and Cheng, 2009).

This research focuses on optimization of organosolv pre-treatment (clean fractionation), using solvent mixture components suggested by NREL (Black et al., 1998), applied to switchgrass as a feedstock for cellulose and lignin production. The treatment was statistically optimized for solvent composition and processing conditions (catalyst, temperature and time) using Response Surface Methodology. The fractionation process was evaluated based on producing the highest glucose yield, and maximizing amount of extracted lignin and hemicellulose.

1.1. Sample preparation and chemicals

SG biomass was harvested from a local farm near Brookings, South Dakota. Before any processing, the SG was air dried, than ground in a hammer mill (Speedy King, Winona Attrition Mill Co, MN, USA) using an 8-mm sieve, then ground to pass through a 1-mm screen (Thomas–Wiley Laboratory Mill, Model 3375-E15, Thomas Scientific, USA). The moisture content of the ground SG samples was measured according to NREL/TP-510-42621 (Sluiter et al., 2008a). Composition analysis was determined according to NREL procedure NREL/TP-510-42618 (Sluiter et al., 2008c). It was determined that the ground SG contained: 37.38 ± 1.11% glucose, 18.32 ± 0.54% xylose, 3.03 ± 0.08% arabinose, 1.68 ± 0.33% galactose, 20.65 ± 0.66% acid insoluble lignin (AIL), 1.66 ± 0.21% acid soluble lignin (ASL), 2.99 ± 0.07% ash, 4.47 ± 0.11% water soluble extractives and 3.17 ± 0.11% ethanol soluble extractives (all calculated per dry matter).

Sulfuric acid stock solution (72%) (R81916001A, Fisher Scientific, Ricca Chemical) was used to prepare the 0.005 M mobile phase for high performance liquid chromatography (HPLC) analysis. Sugar and by-product chromatography standards included D-(+)-cellobiose (108460250, Acros Organics), D-(+)-glucose (G8270, Sigma), D-(+)-xylose (141001000, Acros Organics), D-(−)-arabinose (161450250, Acros Organics), D-(+)-galactose (150611000, Acros Organics), D-(+)-mannose (150600250, Acros Organics) acetic acid (242853-500, Sigma Aldrich), 2-furfuraldehyde (185914, Sigma–Aldrich), and 5-hydroxymethyl-2-furfuraldehyde (HMF, W501808-25, Sigma Aldrich Fine Chemicals).

1.2. Experimental design

The pulping experiments were performed using a central composite design (CCD). The effects of four factors, including digestion time (10–50 min), temperature (110–160 °C), catalyst concentration (0.21–0.93% w/w) and organic solvent mixture composition (7–43% MIBK w/w) on glucose yield, delignification extent and xylan removal were tested. The experimental factors and corresponding levels are presented in Table 1, whereas the experimental design is presented in Table 2. The factors' variation ranges were based on our previous work employing the clean fractionation process for prairie cordgrass pretreatment (Brudecki et al., 2012). The experimental region included 16 factorial design points, eight axial points ($\alpha = 2$), and six replicate center points. Two replications were performed for all factorial and axial design points. Organic solvent mixture was composed of methyl isobutyl ketone (MIBK), ethanol (EtOH) and water (H_2O) in different proportions. A ternary mixture phase diagram was used to set the proportions of the components (MIBK/EtOH/H_2O) (Solimo et al., 1989). Components ratios were chosen such that the mixture remained as a single phase; therefore ethanol content (which was mixability agent) was 5% higher than the phase transition line for each solvent mixture.

Table 1
Experimental design factors and corresponding values.

Coded factor levels	Factor 1 time (min)	Factor 2 temperature (°C)	Factor 3 catalyst – H_2SO_4 (%w/w of solvent)	Solvent composition		
				Factor 4 MIBK (%w/w)	Water (%w/w)	Ethanol (%w/w)
−α	10	120	0.21	7	63	30
−1	20	130	0.39	16	50	34
0	30	140	0.57	25	40	35
+1	40	150	0.75	34	31	35
+α	50	160	0.93	43	25	32

1.3. Clean fractionation (CF)

Clean fractionation separates lignocellulosic biomass into cellulose, hemicellulose and lignin fractions. A simplified flowchart of the laboratory-scale CF process is presented in Fig. 1. Methyl isobutyl ketone and ethanol used for digestion were purchased from Fisher Scientific. The instrumentation used for digestion was custom-made, consisting of an aluminum heating block with six pockets holding stainless steel reactors. Each reactor (capacity ~250 mL) was sealed with a screw cap utilizing a Teflon ring as the seal. Screw caps were equipped with a thermocouple, pressure gauge and quick connector attached to the top. Temperature profiles were monitored by Lab View 8.2 software and a data acquisition device (Omega, OMB-DAQ-56). For each experiment 7 g (DM) of SG and 100 g of organic solvent mixture with catalyst were used.

After the pulping process, the reactors were removed from the heating block and quenched to ambient temperature in a water bath. The reaction mixture was filtered through polypropylene cloth, separating solids from liquor. The reactors were rinsed with 25 mL of water and the same rinsate was used to "first wash" the solid fractions to remove residual solvent. The liquor and rinsate were transferred to a separatory funnel. The rinsate combined with the liquor induced phase separation into organic and aqueous. The pulp was washed with additional water (around 2 L) in order to remove solvent residues. Washed cellulose solid fraction (output I) was collected and kept frozen for subsequent analysis and enzymatic hydrolysis. The two layers in the separatory funnel were allowed to separate over an hour, and then released, resulting in the bottom aqueous fraction (output II, water-based, rich in hemicellulose) and the top organic fraction (output III, MIBK-based, rich in lignin). The organic fraction was evaporated and dried to remove the solvent, and the solid air-dried lignin fraction was weighed.

1.4. Mass balance and fraction composition

Cellulose-, hemicellulose-, and lignin-rich fractions were weighed to perform mass balances on the process. The total solid (TS) content was measured in the cellulose solid fraction in order to determine the pulp yield and the amount of extracted solids from the SG biomass. Compositional analyses (sugar and AIL content) of dried and ground cellulose and dried lignin samples were performed by acid hydrolysis according to NREL procedure NREL/TP-510-42618 (Sluiter et al., 2008c). The sugar content in acid hydrolysates was analyzed using an HPLC system (Agilent Technologies System 1200 with Biorad Aminex 87H Column), with an RID detector, 0.005 M H_2SO_4 mobile phase at a flow rate of 0.6 mL/min at 65 °C, and 20 μL sample injection, as designated in NREL/TP-510-42623 (Sluiter et al., 2008b). The aqueous fractions obtained from clean fractionation phase separation were analyzed using the same procedure; however, an aliquot of 10 μL was injected into the HPLC system for each analysis. One analysis ($n = 1$) was performed for each replicate.

Table 2
Central composite experimental design matrix.

Run	Factor 1 A:time (min)	Factor 2 B:temp (°C)	Factor 3 C:catalyst (%w/w)	Factor 4 D:MIBK (%w/w)	Factor 1 A:time (min)	Factor 2 B:temp (°C)	Factor 3 C:catalyst (%w/w)	Factor 4 D:MIBK (%w/w)
	Actual				Coded			
1	20	130	0.75	16	−1	−1	1	−1
2	40	150	0.39	34	1	1	−1	1
3[a]	30	140	0.57	25	0	0	0	0
4	40	150	0.75	34	1	1	1	1
5	30	140	0.57	7	0	0	0	−2
6[a]	30	140	0.57	25	0	0	0	0
7[a]	30	140	0.57	25	0	0	0	0
8	30	120	0.57	25	0	−2	0	0
9	50	140	0.57	25	2	0	0	0
10	40	150	0.39	16	1	1	−1	−1
11	20	150	0.39	34	−1	1	−1	1
12	40	130	0.75	16	1	−1	1	−1
13	40	150	0.75	16	1	1	1	−1
14	20	150	0.75	34	−1	1	1	1
15	30	140	0.21	25	0	0	−2	0
16	20	150	0.39	16	−1	1	−1	−1
17	20	130	0.75	34	−1	−1	1	1
18	40	130	0.75	34	1	−1	1	1
19	40	130	0.39	16	1	−1	−1	−1
20	20	130	0.39	16	−1	−1	−1	−1
21	30	160	0.57	25	0	2	0	0
22[a]	30	140	0.57	25	0	0	0	0
23[a]	30	140	0.57	25	0	0	0	0
24	40	130	0.39	34	1	−1	−1	1
25	30	140	0.57	43	0	0	0	2
26	20	150	0.75	16	−1	1	1	−1
27	10	140	0.57	25	−2	0	0	0
28	20	130	0.39	34	−1	−1	−1	1
29	30	140	0.93	25	0	0	2	0
30[a]	30	140	0.57	25	0	0	0	0

[a] Replicates of center points in experimental design.

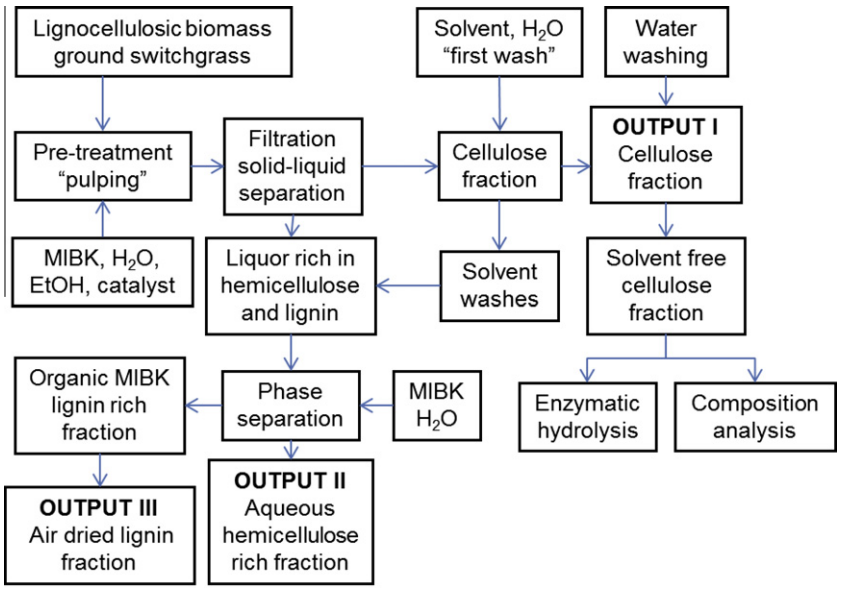

Fig. 1. Flowchart of lab-scale clean fractionation (CF) process.

1.5. Enzymatic hydrolysis of cellulose fraction

The effectiveness of the pre-treatment was evaluated based on the enzymatic hydrolysis glucose yield. Enzymatic hydrolysis was performed under the same conditions for all pulp samples obtained from different treatment conditions, to study the effects of processing conditions on the cellulose digestibility. Hydrolysis was carried out according to NREL protocol NREL/TP-510-42629 (Selig et al., 2008). Pulp samples (output I obtained from the CF process; Fig. 1) equal to 0.3 g of DM, 0.1 M sodium citrate buffer (pH = 4.8) and water were loaded into the scintillation vials. The enzymes were added last, since the hydrolysis reaction was initiated by the addition of the enzymes. Cellulase and β-glucosidase enzymes were provided by Novozymes. Dosage of the cellulase (NS50013, activity 70 FPU/g, FPU = Filter Paper Unit) enzyme was equal to 15 FPU/g DM and β-glucosidase (NS50010, activity 250 CBU/g, CBU = Cellobiase unit) was equal to 60 CBU/g DM (Karunanithy and Muthukumarappan, 2010). Hydrolysis was carried out at 50 °C for 72 h and 150 rpm in an incubated shaker (Fisher Thermo Scientific, Incubated Tabletop Orbital Shaker, model 420). All hydrolysis runs were performed in duplicate. Samples of hydrolysates were collected and filtered through a 0.2 μm nylon syringe filter. Concentrations of sugars, organic acids, and by-products in the enzymatic hydrolysates were measured using an HPLC system, as described before and as designated in NREL/TP-510-42623 (Sluiter et al., 2008b).

1.6. Statistical analyses

Statistical analyses were performed using Design Expert (version 8.0.6., Minneapolis, MN). Multiple linear regression (MLR) method was used to determine equations for response models. Backward elimination of model terms was employed, and only the terms with the statistically significant coefficients (greater than or equal to 95% confidence level) were included in the final regression models. In order to improve the statistical analysis and/or diagnostic plots, some of the data were transformed. A Box–Cox plot tool was used to determine the most appropriate power transformation to apply to the response data. The lack of fit parameter and coefficients of determination were taken into consideration to assess model fit to the data. Since R^2 can be artificially inflated by adding terms to the model, the adjusted and predicted R^2 are presented as well. Predicted and adjusted R^2 values were assessed to be within 0.2 of each other.

2. Results and discussion

2.1. Mass balance

Measurements of total solids in the cellulose solid fraction were used to perform the mass balances. The difference between input and output of total solids in the solid fractions was assumed to be solubilized in the aqueous and organic fractions. The mass balance of total solids of the CF process is presented in Table 3. The pulp yield, expressed by the ratio of solids recovered in the pulp to the solid input, ranged between 33% and 51%. The pulp yields depended on the pre-treatment severity parameters, associated mainly with levels of catalyst and temperature. Leaching of biomass components was more effective with high levels of catalyst and temperature (Fig. 2a), while variation in time and solvent mixture composition did not affect pulp yields as much (therefore not presented). The same tendency was observed when working with clean fractionation of prairie cordgrass (Brudecki et al., 2012). The regression model and resulting coefficients of determination for pulp yield are presented in Table 4. The only significant quadratic term in this model was for catalyst concentration; however, linear terms for all factors and two interactions terms were significant as well.

Dried lignin fraction yield ranged between 0.85 g and 3.79 g (Table 3). The lowest dried lignin fraction outputs were observed for runs 20 and 5, associated with low levels of all the factors (run 20) and lowest level of MIBK content in the solvent mixture (run 5). The highest dried lignin fraction outputs were observed for the runs treated with high MIBK content in the organic solvent mixture (Table 3). The MIBK factor was also observed to be the most influential on this response, while an increase in catalyst concentration resulted in only a small increase of dried lignin fraction output (Fig. 2b). Time and temperature did not affect dried lignin output response much. A small negative trend was observed between dried lignin fraction outputs and pulp yields ($R^2 = -0.41$). The regression model and resulting coefficients of determination for dried lignin fraction output are presented in Table 4.

Table 3
Mass balances of CF treatments.

Run	Switchgrass biomass DM[b] input (g)	Output I		Leached fraction of biomass to the solvent mixture (g)	Output II	Output III
		DM[b] output in pulp (g)	Pulp yield (%)		DM[b] output in hemicellulose fraction (g)	Dried lignin fraction (g)
1	7.00	3.24	46.25	3.76	2.46	1.19
2	7.00	2.78	39.77	4.22	1.45	3.16
3[a]	7.00	2.83	40.44	4.17	2.14	1.86
4	7.00	2.31	33.02	4.69	1.82	2.83
5	7.00	3.06	43.71	3.94	2.43	0.91
6[a]	7.00	2.89	41.23	4.11	2.18	1.86
7[a]	7.00	2.99	42.72	4.01	2.12	1.82
8	7.00	3.24	46.23	3.76	1.28	2.10
9	7.00	2.80	40.03	4.20	2.16	2.06
10	7.00	2.63	37.59	4.37	2.50	1.35
11	7.00	2.48	35.49	4.52	1.39	3.00
12	7.00	3.07	43.92	3.93	2.46	1.15
13	7.00	2.72	38.81	4.28	2.57	1.52
14	7.00	2.36	33.78	4.64	1.59	3.04
15	7.00	3.55	50.75	3.45	2.06	1.50
16	7.00	2.80	39.95	4.20	2.34	1.32
17	7.00	2.96	42.29	4.04	1.43	3.05
18	7.00	2.84	40.51	4.16	1.40	3.45
19	7.00	3.29	47.01	3.71	2.10	1.08
20	7.00	3.55	50.65	3.45	2.22	0.85
21	7.00	2.32	33.08	4.68	1.87	2.52
22[a]	7.00	2.87	41.07	4.13	2.21	1.83
23[a]	7.00	2.96	42.28	4.04	2.16	1.78
24	7.00	3.22	46.06	3.78	1.29	2.90
25	7.00	2.69	38.41	4.31	1.11	3.79
26	7.00	2.59	36.95	4.41	2.48	1.36
27	7.00	2.90	41.36	4.10	2.04	2.11
28	7.00	3.47	49.63	3.53	1.11	2.72
29	7.00	2.58	36.86	4.42	2.01	2.24
30[a]	7.00	3.03	43.29	3.97	2.07	1.70

[a] Replicates of center points in experimental design.
[b] DM denotes dry matter basis.

2.2. Fraction composition

Glucan, xylan and AIL content in the pulps are presented in Table 5. Glucan content in the pulp fraction ranged between 78% and 94%. Runs performed at high temperature and high catalyst concentration resulted in significant leaching of glucose to the organic solvent mixture (e.g., runs 4, 14 and 21 resulted in 20–21% glucan removal, or 78–79% glucan remaining in the pulp). The regression model and resulting coefficients of determination for glucan remaining in the pulp is presented in Table 4. Time was found to be an insignificant factor on this response. A quadratic term for temperature was found to be significant, and temperature variations were found to be highly influential on this response (Fig. 2c). Catalyst concentration and MIBK quantity were found to be significant; however, each had much smaller influence on the glucan content in the pulp.

Xylan remaining in the pulp was quite low; it ranged between 0% and 14% of initial xylan content in raw SG. This indicates high xylan removal from SG. Variations of temperature and catalyst concentration were found to be the most influential on xylose content remaining in the pulps. Additionally, an increase of digestion temperature and catalyst concentration resulted in higher xylan removal from the SG. Treatments with high temperature and high catalyst concentration resulted in complete xylan removal from the SG (Fig. 2d). Increase of digestion time and MIBK content in the solvent mixture also improved xylan removal; however, only to a small extent, and did not have as big an impact as the two other factors. The final regression equation and associated coefficients of determination for xylan remaining in the pulp are presented in Table 4. Linear terms for all the factors, an interaction term between temperature and catalyst concentration, and quadratic terms for catalyst concentration and MIBK content in the solvent mixture were found to be significant for this model.

Acid insoluble lignin (AIL) remaining in the pulp ranged between 7% and 25% (% of initial lignin content in the raw SG) (Table 5). Delignification extent is presented in Table 5 as well, and it was calculated by subtracting AIL remaining in the cellulose fraction after the CF process from the initial AIL content in the raw SG. Raising digestion temperature, catalyst, and MIBK concentration resulted in an increased AIL removal from SG (less AIL remaining in the pulp), while time variation produced a parabolic response for AIL removal from SG, as presented in Fig. 2e and f. The smallest content of AIL in the pulp was observed for run 21 (6.62%), which had been treated with the highest temperature (160 °C). However, several other runs resulted in similarly low pulp AIL content (e.g., runs 2, 4, 9, 11, and 14) which produced pulp with less than 8% AIL content. These runs were associated with high levels of the processing factors. The highest content of AIL in the pulp (also reflecting the smallest delignification) was found for runs 5 (21.83%), 15 (24.56%) and 20 (24.71%). The small delignification extent in these runs was associated with the lowest level of MIBK content in the solvent mixture (run 5), smallest level of catalyst concentration (run 15), and low levels of all these factors (run 20), respectively. The regression equation and associated coefficients of determination for AIL remaining in the pulp are presented in Table 4. Linear terms for all the factors, an interaction term between temperature and catalyst concentration, and quadratic terms of time and temperature were found to be significant for this model. A strong positive correlation (R^2 = 0.84) was observed between AIL and xylan remaining in the pulp, which confirms parallel lignin and xylan removal. The AIL removal from SG (i.e. run 25 with 89%) was found to be higher when compared to lignin removal from prairie cordgrass

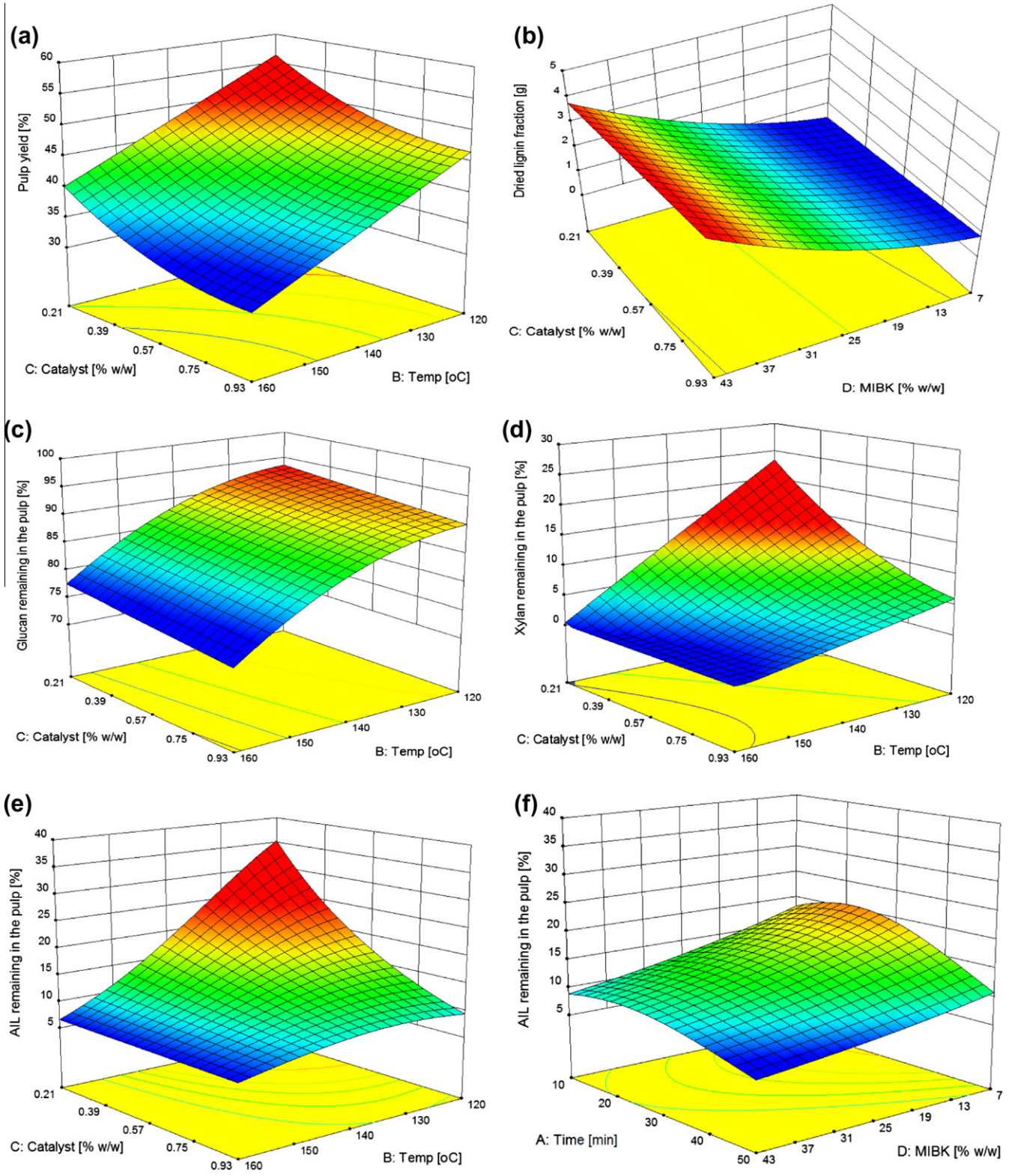

Fig. 2. Response surface plots of dependent variables: (a) pulp yield, (b) air dried lignin fraction, (c) glucan (d) xylan and (e and f) AIL remaining in the pulp vs. significant factors.

(PCG) (i.e. 79%) using similar clean fractionation conditions (Brudecki et al., 2012). Also, higher delignification was observed in the case of SG when compared to PCG, using ethyl acetate as a lignin solvent in the clean fractionation solvent mixture instead of MIBK (Cybulska et al., 2012b). Also, delignification of SG (89%) was higher than delignification of corn stover (74%) in related work, even though catalyst concentration employed for corn stover clean fractionation was twice higher (Ishizawa et al., 2009). However, when comparing the results to similar work on clean fractionation performed on hardwoods (i.e. poplar and oak group), similar

Table 4
Resulting regression models.

Regression model final equations in terms of actual factors, where A = time, B = temperature, C = catalyst and D = MIBK	R^2	Adjusted R^2	Predicted R^2
Pulp yield (%) = + 147.53026 − 1.30128 A − 0.67436 B − 23.36944C + 0.12128, D + 8.96871E-003AB − 0.46608CD + 18.86438C^2	0.9161	0.8894	0.8011
Dried lignin fraction (g) = + 15.68546 − 0.033911A − 0.27440B + 7.13657C + 0.14622D − 0.046528BC − 9.51389E-004BD + 6.21429E-004A^2 + 1.19018E-003B^2 + 1.60053E-003D^2	0.9750	0.9638	0.9226
Glucan remaining in the pulp (%) = − 20.86465 + 2.03147B − 4.70842 C − 0.12563D − 8.69190E-003B^2	0.8159	0.7839	0.6926
Xylan remaining in the pulp (%) = +116.80072 − 0.099165A − 0.69627B − 117.38282C + 0.29517D + 0.57569BC + 21.11255C^2 − 7.97491E-003D^2	0.8966	0.8604	0.7420
Ln(AIL remaining in the pulp (%)) = −0.95775 + 0.037434A + 0.11238B − 7.24766C − 0.020229D + 0.045004BC − 8.46059E-004A^2 − 5.85368E-004B^2	0.9248	0.9008	0.8507
Glucose in the aqueous fraction (%) = − 14.77149 + 0.017785 + 0.11536B + 8.75030C − 0.057486D − 4.75127C^2	0.9317	0.9175	0.8792
Xylose in the aqueous fraction (%) = −1367.85299 + 5.72721A + 16.66209B + 599.52764C − 1.36144D − 0.033503AB − 1.74502AC − 2.94962BC − 0.048338B^2 − 100.84295C^2	0.8875	0.8369	0.6116
Enzymatic hydrolysis glucose yield (%) = −935.86747 + 1.07444A + 11.83256B + 530.63821C + 1.37824D − 1.55663AC − 2.37907BC − 1.42548CD − 0.036859B^2 − 86.79597C^2 − 0.011856D^2	0.9165	0.8700	0.7595

Table 5
Composition of the pulps and aqueous fractions; enzymatic hydrolysis glucose yields and efficiencies obtained from clean fractionation of raw switchgrass (SG).

Run	Glucan remaining in the pulp (%)[b,e]	Xylan remaining in the pulp (%)[c,e]	AIL remaining in the pulp (%)[d,e]	Delignification extent = AIL extracted from SG biomass (%)[d,e]	Glucose in the aqueous fraction (%)[b]	Xylose in the aqueous fraction (%)[c]	Enzymatic hydrolysis glucose yield (%)	Enzymatic hydrolysis efficiency (%)
1	93.72	9.85	17.75	82.25	3.15	59.60	94.79	101.15
2	93.89	0.00	7.21	92.79	3.24	45.03	99.42	105.89
3[a]	89.44	5.03	13.52	86.48	4.13	63.91	95.26	97.76
4	77.65	0.00	6.87	93.13	5.91	34.96	81.68	105.19
5	89.31	3.35	21.83	78.17	5.24	80.11	90.21	101.00
6[a]	87.61	4.16	12.68	87.32	3.97	60.82	93.44	106.65
7[a]	86.41	5.55	12.41	87.59	3.63	55.30	91.55	105.95
8	90.00	10.48	18.50	81.50	2.28	42.57	75.23	83.59
9	89.28	0.30	6.88	93.12	4.37	62.55	94.79	106.18
10	84.63	1.18	10.37	89.63	5.44	77.09	91.02	107.55
11	80.29	0.70	7.98	92.02	3.67	45.28	90.05	112.16
12	89.96	7.25	12.73	87.27	3.78	66.25	94.06	104.56
13	83.72	0.00	10.20	89.80	5.35	50.37	92.41	110.38
14	78.95	0.00	6.97	93.03	4.63	43.34	85.47	108.25
15	91.95	13.99	24.56	75.44	2.02	31.13	74.51	81.03
16	84.12	3.32	13.40	86.60	4.81	71.20	85.62	101.78
17	89.83	5.64	11.46	88.54	2.35	35.77	93.37	103.94
18	91.27	2.67	9.31	90.69	2.47	36.43	91.92	100.71
19	89.94	11.23	18.43	81.57	2.52	50.70	85.65	95.23
20	93.02	13.90	24.71	75.29	2.24	42.31	72.79	78.25
21	77.61	0.00	6.62	93.38	6.45	41.77	82.54	106.35
22[a]	85.75	3.50	13.19	86.81	4.03	63.05	96.58	112.63
23[a]	85.27	6.28	14.68	85.32	3.95	59.21	94.51	110.84
24	89.45	9.40	13.66	86.34	1.82	30.22	86.94	97.19
25	87.43	0.00	11.50	88.50	2.95	33.96	89.37	102.22
26	84.77	0.00	11.83	88.17	6.03	77.56	91.74	108.22
27	83.72	5.03	14.45	85.55	3.36	54.36	89.43	106.82
28	90.89	12.64	19.60	80.40	1.39	22.25	75.06	82.59
29	84.11	0.00	8.40	91.60	4.96	62.65	90.25	107.30
30[a]	89.62	4.44	11.94	88.06	3.78	55.98	98.32	109.71

[a] Replicates of center points in experimental design.
[b] Percent of initial glucose content in raw SG biomass.
[c] Percent of initial xylose content in raw SG biomass.
[d] Percent of initial AIL content in raw SG biomass (AIL denotes acid insoluble lignin).
[e] Results obtained by acid hydrolysis.

delignification extents were obtained (90% and higher delignification, which depends on the treatment conditions) (Bozell et al., 2011).

Glucose and xylose content in the aqueous fractions are presented in Table 5. Glucose content in the aqueous fractions ranged between 1% and 7% of initial glucose content in the raw SG. It is desired to keep the glucose concentration in the liquid fraction low, since it reduces fermentation potential of the cellulosic pulp. The regression equation and associated coefficients of determination for glucose content in the aqueous fractions is presented in Table 4. Linear terms for all the factors, and a quadratic term for the catalyst concentration were found to be significant for this model. An increase in digestion temperature, time, and concentration of the acid resulted in a slight increase in glucose content in the aqueous fraction, while a MIBK content increase had the reverse effect, resulting in a decline of glucose content in the aqueous fraction (Table 5). High MIBK content resulted in a partial glucose separation to the organic fraction (discussed more later). Less than 2%

of the initial glucose content in the raw SG was degraded to HMF, and measured in the aqueous fractions.

Xylose content in the aqueous fractions ranged between 22% and 80%. Since the main component of the hemicellulose fraction is xylose (more than 75%), xylose measurements reflect the degree of the hemicellulose depolymerization and removal to the aqueous fraction. The highest amount of xylose detected in the aqueous fractions was observed for run 5 (80% of initial content of xylose in the raw SG), which was performed with the lowest MIBK content (7%) in the solvent mixture. However, several other runs resulted in a similarly high xylose separation to the aqueous fractions (e.g., runs 10, 16 and 26 resulted in more than 70% xylose yield; these runs were performed at high temperature (150 °C) and low level (16%) of MIBK content in the solvent mixture). On the other hand, the lowest xylose content in the aqueous fraction (22%) was observed for run 28, performed at low levels of all processing factors except MIBK, which was set to a high level in this run. The regression model and resulting coefficients of determination for xylose released to the aqueous fraction is presented in Table 4. Linear terms for all processing factors, interactions terms among time, temperature and catalyst concentration, and quadratic terms for temperature and catalyst concentration were found to be significant for this model. A relatively small predicted coefficient of determination was obtained for this model (Pred-R^2 = 0.61). Catalyst and temperature changes resulted in a parabolic response for the xylose content in the aqueous fraction (Fig. 3a). Increase of the MIBK content in the solvent mixture had a negative effect on the xylose content in the aqueous fractions, and resulted in decline of xylose content in aqueous fraction (Fig. 3b). Time did not have much influence on the xylose content in the aqueous fractions, only a slight increase of the xylose content was observed with an increase of digestion time (Fig. 3b). No furfural (degradation product of xylose) was detected in the aqueous fractions.

Complete drying of the lignin fraction was not achieved for all the samples, even though prolonged drying was employed. This phenomenon was especially observed in samples that were treated with a high content of MIBK in the organic solvent mixture. Small quantities of MIBK and ethanol solvent were still detected in the dried lignin fractions after drying. It was presumed that small quantities of the solvent were entrapped or condensed in the structure of the material while drying. It has been noted elsewhere that MIBK can be difficult to evaporate (Hyman et al., 2007). High AIL content was measured in the dried lignin fractions for most of the samples. Interestingly, samples treated with 16% or less MIBK content in the solvent mixture resulted in the lowest AIL content (49–85% of initial AIL in raw SG) in the dried lignin fractions. However, the smallest catalyst concentration in run 15 also produced relatively low AIL in the dried lignin fraction (72% on initial AIL in raw SG). Runs with high MIBK content in the solvent mixture produced more than 100% (% of initial AIL content in the raw SG) of AIL in the dried lignin fractions. Incomplete drying of the organic fractions is probably one of the reasons for the inflated AIL content in the dry lignin fractions since dried lignin weight is a coefficient in the calculations. Other explanations could include MIBK condensation with lignin functional groups, or pseudo lignin formation. As presented by other authors, degraded sugars and low molecular weight phenolics can react with lignin to yield insoluble condensation products or be entrapped in the dried lignin fraction and measured as "lignin" (Chandra et al., 2007).

Inflated lignin content after the ethanol and acetone organosolv pre-treatment of lignocellulosic biomass was also reported by other authors (Huijgen et al., 2010, 2012; Kim and Pan, 2010). A maximum of 3% of the initial glucose in the raw SG was detected in the dried lignin fraction. This confirms small cross-contamination of dried lignin fractions with glucose. Small quantities of furfural and HMF were also detected in the dried lignin fractions; maximum detection was equivalent to 5% of the initial xylose and glucose content in the raw SG. The samples treated with high MIBK content in the solvent mixture resulted in the highest furfural and HMF content in the dried lignin fractions, which is in agreement with other work focused on the production and extraction of HMF using phase modifiers (Román-Leshkov et al., 2006). Up to 50% of initial xylose was detected in the dried lignin fractions; runs with 34% and 43% of MIBK content in the solvent mixture resulted in the dried lignin fractions with high xylose content. Runs with a medium level of MIBK content in the solvent mixture (25%) resulted in up to 20% of xylose content in dried lignin fractions. Low xylose content in the dried lignin fractions (0–5% of initial xylose in raw SG) was observed for all the runs with 16% or less MIBK content in the solvent mixture. These results confirm previously observed trend in this work, indicating that an increase of MIBK content in the solvent mixture results in a decrease of xylose content in the aqueous fraction, in favor of xylose transfer to the or-

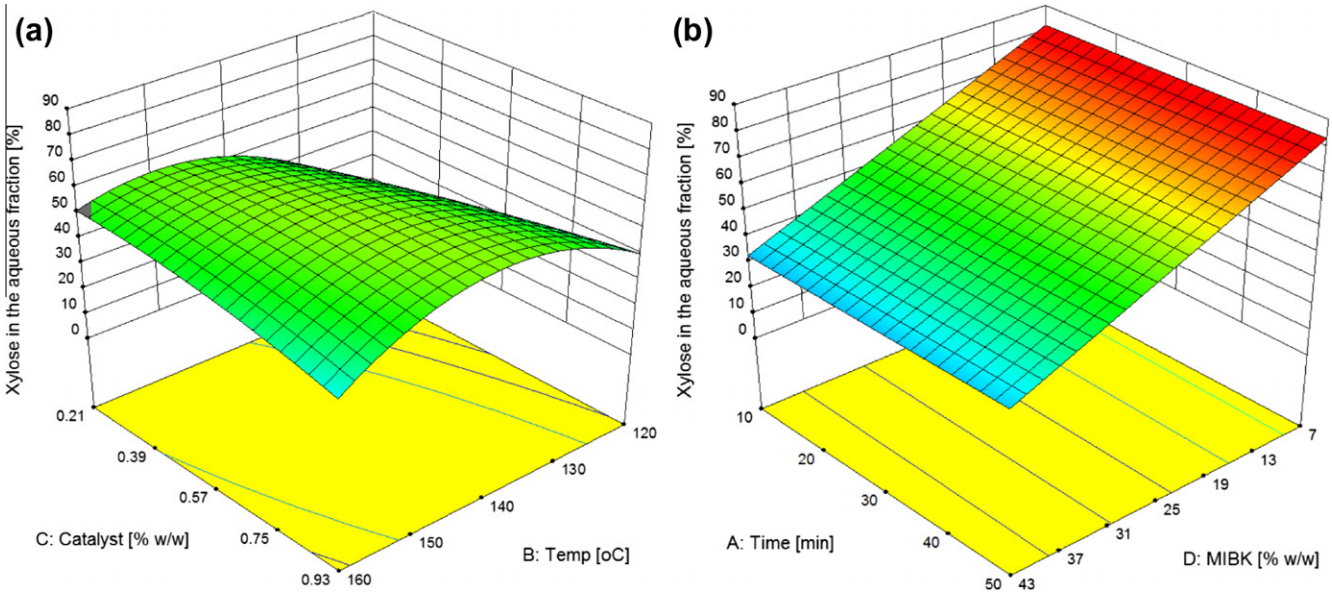

Fig. 3. Response surface plots of xylose content in the aqueous fraction.

ganic phase. Analysis of sugars in the aqueous and organic fractions confirmed the distribution of xylose between phases, and the affinity of xylose to the organic phase. Interestingly, it was also observed that a high level of MIBK content combined with a low level of catalyst favored separation of xylose to the organic fraction. Based on these results, it can be concluded that separation of xylose to the organic fraction increased with an increase in MIBK content in the solvent mixture and a decline in the catalyst concentration. Additional multiple extractions would be recommended for some of the runs in order to separate xylose to the aqueous fraction more selectively. Another approach could be reducing MIBK content in the solvent mixture. As discussed previously, run 5 with only 7% of MIBK in the solvent mixture resulted in 80% xylose in the aqueous fraction, but it produced one of the smallest delignification rates (78% of AIL removal) (Table 5).

Following the biorefinery concept, lignin and hemicellulose fractions obtained from the clean fractionation process should be converted into value-added products. For example, lignin containing high levels of guaiacyl units can be converted to vanillin by nitrobenzene oxidation. A recent study performed on lignin extracted from prairie cordgrass, switchgrass and corn stover, using modified clean fractionation (using an ethyl acetate, ethanol and water solvent mixture), showed high potential in vanillin production. Relatively high yields of vanillin were obtained in the case of all lignin types (approx. 40–60% w/w, calculated per initial lignin input weight), however only 39% w/w was obtained in the case of SG originated lignin (Cybulska et al., 2012b).

Detailed characterization of lignin and hemicellulose fractions is recommended as a pre-step for specific product development from these two fractions. In addition, a purification and separation of hemicellulose and lignin fractions should be the subject of future research. Moreover, a recovery of the solvents should also be investigated since overall economics of the organosolv process is highly dependent on solvent consumption.

2.3. Enzymatic hydrolysis of cellulose fraction

The glucose yield was calculated as the percentage of glucose measured in the enzymatic hydrolysate divided by the initial content of glucose in the raw SG. Hydrolysis efficiency (expressed as a percent ratio), reflecting cellulose digestibility, was calculated as the ratio of the glucose input (measured by acid hydrolysis) to the glucose output in the enzymatic hydrolysate. Glucose yield and enzymatic hydrolysis efficiency are presented in Table 5. Enzymatic hydrolysis performed on untreated raw SG resulted only in glucose concentration equal to 1.7 mg/mL equivalent to 15% glucose yield. Glucose concentrations measured in the enzymatic hydrolysates of pulps obtained from fractionation ranged between 15 and 29 mg/mL. The concentration of glucose stabilized after 48 h, indicating that enzymatic hydrolysis could be carried out using a shorter time. Most of the runs resulted in concentrations higher than 20 mg/mL; only runs 8, 15, 20 and 28 produced lower concentrations (16–18 mg/mL). These runs were performed at the lowest temperature (run 8), lowest catalyst concentration (run 15), or a combination of low levels of temperature, catalyst concentration and time (runs 20 and 28). These same runs produced some of the highest pulp yields (46–50%) and the lowest glucose yields (73–75%) (Tables 3 and 5). The highest glucose yield was determined for run 2 (99.42%). Runs 1, 12, and four of the replicate center points (runs 3, 22, 23, and 30) produced a glucose yield of more than 94%. The regression model and resulting coefficients of determination for glucose yield are presented in Table 4. Linear terms for all the factors, interactions terms among catalyst concentration and the other factors, and quadratic terms for temperature, catalyst concentration, and MIBK content were found to be significant for this model. Catalyst concentration, temperature and MIBK content variations resulted in a parabolic response for the glucose yield (Fig. 4). Temperature and catalyst concentrations were found to be the most influential on glucose yield response. MIBK had a much smaller effect on this response, and only slight increase in glucose yield was observed with an increase of digestion time (Fig. 4b). The highest glucose yields obtained in this work (close to 100%) were found to be higher than those reported in a similar study using prairie cordgrass as a feedstock (max. 84% glucose yield) (Brudecki et al., 2012). In a related study, ethanol organosolv pretreated switchgrass yielded 92% recovery of the glucan present in the untreated switchgrass after 72 h of enzymatic hydrolysis, confirming the effectiveness of the organosolv pre-treatment (Cateto et al., 2011).

Enzymatic hydrolysis efficiencies were found to be very high (Table 5). Almost all of the runs resulted in more than 100% enzymatic hydrolysis efficiency. This indicates that all of the glucose

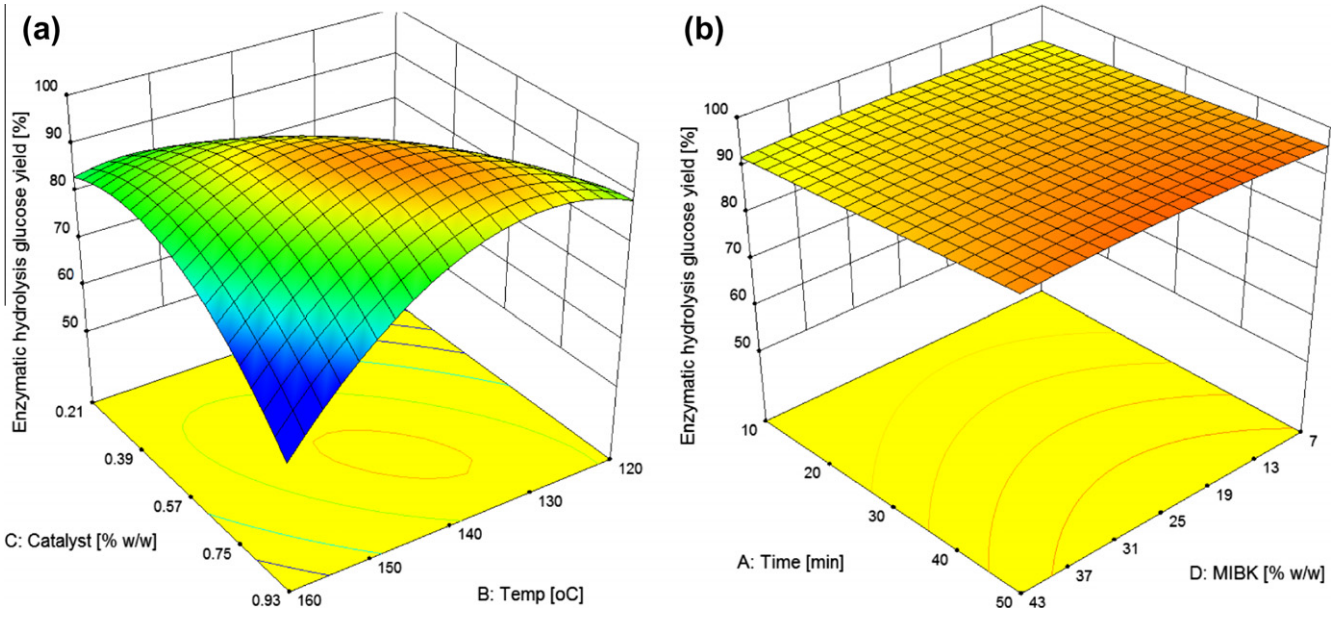

Fig. 4. Response surface plots of enzymatic hydrolysis glucose yield.

measured in the pulp fractions by acid hydrolysis was accessible for enzymatic action. Efficiencies higher than 100% also indicate that enzymatic hydrolysis was more effective for cellulose digestibility than acid hydrolysis. It can be assumed that long enzymatic hydrolysis time allows for complete cellulose depolymerization. Only four runs resulted in relatively low enzymatic hydrolysis efficiencies, between 78% and 84% (runs 8, 15, 20 and 28). As mentioned before, these runs produced the lowest glucose concentrations in the enzymatic hydrolysates, and therefore low hydrolysis efficiencies. No statistical model is presented in Table 4 for enzymatic hydrolysis efficiency, due to low coefficients of determination. High hydrolysis efficiencies are in agreement with results previously reported for prairie cordgrass treated by clean fractionation (Brudecki et al., 2012).

The extent of cellulose digestibility was higher when compared to related work on clean fractionation of corn stover (approx. 70% when using similar treatment conditions) (Ishizawa et al., 2009). Cellulose digestibility in this work was also higher than results obtained from other leading pretreatment technologies (e.g., ammonia fiber expansion, dilute sulfuric acid, liquid hot water, lime, and soaking in aqueous ammonia) applied to three cultivars of switchgrass (Kim et al., 2011). For comparative reasons, dried pulp samples were subjected to enzymatic hydrolysis in this study. Although low drying temperatures were applied (40 °C), the digestibility was found to be lower for dried samples when compared to wet samples (average hydrolysis glucose efficiencies among all dried samples was lower by 25% than the wet samples). This can be explained because drying of the pretreated lignocellulose has been reported to reduce enzymatic digestibility as a result of smaller pore volume (Huijgen et al., 2010).

It has been demonstrated by various researchers that enzymatic digestibility of pretreated biomass can be significantly increased due to delignification and hemicellulose removal, resulting in an increase of accessible surface area. Enzyme performance can be highly limited or inhibited by the presence of lignin during hydrolysis (Hsu, 1996; Zhu et al., 2008). This negative effect due to lignin remaining in the pulp was observed in this study as well. Generally, more delignified samples resulted in higher glucose yields (Fig. 5a); however, relatively low coefficient of determination was obtained (R^2 = 0.514). The same trend was observed in previous work using prairie cordgrass as a feedstock with clean fractionation pretreatment; however, the trend was more evident (R^2 = 0.947) (Brudecki et al., 2012). No correlation was observed between enzymatic hydrolysis efficiency and AIL remaining in the pulp (R^2 = −0.016). According to other studies (Chang and Holtzapple, 2000), complete removal of lignin is not mandatory for efficient cellulose digestibility during enzymatic hydrolysis. Cleavage of the bonds between lignin and cellulose results in an increase of the efficiency of the enzymatic hydrolysis, even though lignin may still be present in the material.

Hemicellulose content in the pulp affects the enzymatic hydrolysis efficiency as well. As reported in other studies, hemicellulose removal from the lignocellulosic biomass improves the enzymatic digestibility of the cellulose (Chandra et al., 2007; Mosier et al., 2005). Hemicellulose and lignin removal result in an increased pore volume of the pretreated material, which improves the enzyme penetration and access to the cellulose fibers (Chandra et al., 2007; Mooney et al., 1998). The complex structure of the lignocellulosic biomass and close associations between cellulose, hemicellulose and lignin constitute a physical and chemical barrier for enzyme actions. In this study, it was observed that xylan remaining in the pulp was negatively correlated with glucose yields (Fig. 5b). The same trend was observed in previous work on clean fractionation of prairie cordgrass (R^2 = 0.978) (Brudecki et al., 2012). In this work on switchgrass, relatively low coefficient of determination was produced (R^2 = 0.494); however, harsher treatment conditions were used in this study than in the previous work on prairie cordgrass. No correlation was observed between enzymatic hydrolysis efficiency and xylan remaining in the pulp (R^2 = −0.029). Clean fractionation applied to corn stover showed that xylan removal had more of an impact on the enzymatic digestibility than delignification (Ishizawa et al., 2009). Moreover, the same study revealed that almost complete removal of lignin using acidified sodium chlorite can reduce cellulose accessibility to enzymes due to aggregation of the cellulose microfibrils (Ishizawa et al., 2009). However, another study revealed that removal of lignin was much more favorable than removal of hemicellulose for increasing the enzymatic digestibility when peracetic acid pretreatment was applied to sugarcane bagasse under mild conditions. In this study, xylan was more effectively removed from SG

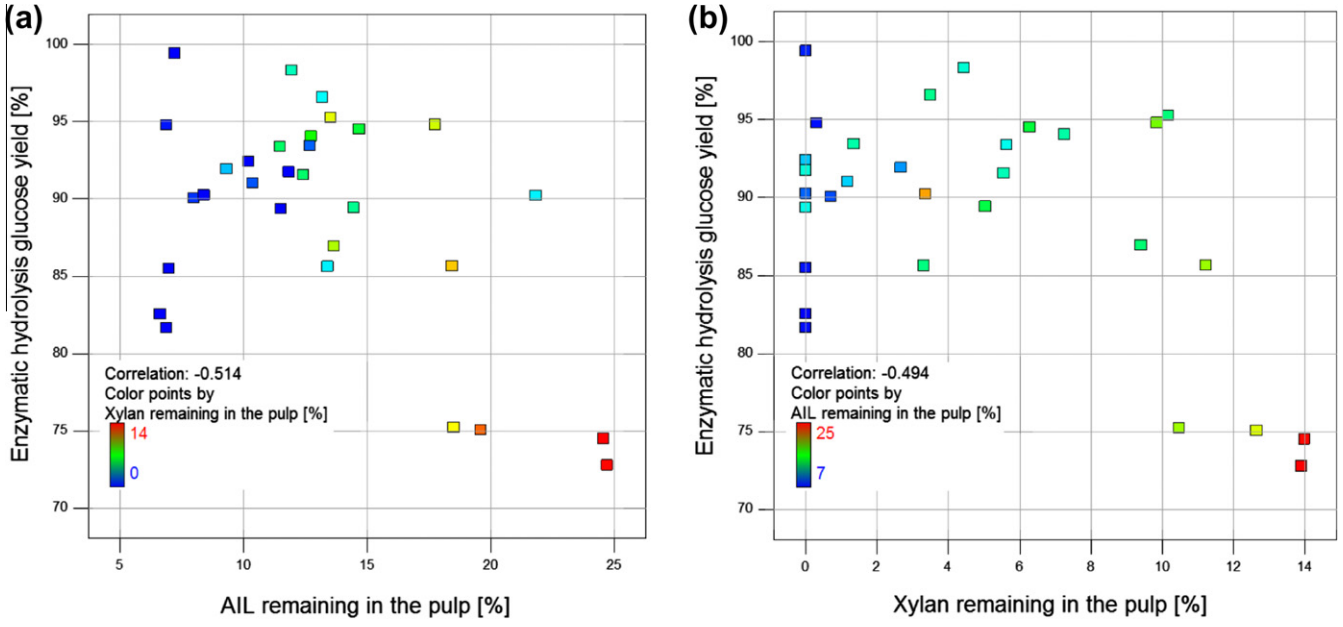

Fig. 5. Effect of (a) AIL removal and (b) xylan removal on enzymatic hydrolysis glucose yield.

than lignin. Samples which resulted in less than 18% of AIL in the pulp (% of initial lignin in the raw SG) produced 80% and higher enzymatic hydrolysis glucose yield (Fig. 5a, Table 5). On the other hand, samples for which xylan removal was below 10% (% of initial xylan in the raw SG) in the pulp produced 80% and higher enzymatic hydrolysis glucose yield (Fig. 5b). Therefore, it appears that high xylan removal may be more important than high lignin removal to obtain high enzymatic hydrolysis glucose yield; however, removal of both lignin and hemicellulose is parallel and plays significant role in hydrolysis efficiency. However other covariates characterizing the pulp structure can also affect the enzymatic hydrolysis including cellulose crystallinity, degree of polymerization, specific surface area and pore volume (Alvira et al., 2010).

2.4. Optimization

Finding the fractionation conditions resulting in the highest glucose yield produced by enzymatic hydrolysis of pulp obtained from the clean fractionation of switchgrass was the main objective of this study. For each specified optimization condition numerical methods provided several potential solutions. Four different optimization alternatives were considered: (alternative 1) highest glucose yield, (alternative 2) highest glucose yield with lowest levels of treatment factors, (alternative 3) highest glucose yield combined with maximum AIL and xylan removal, and (alternative 4) highest glucose yield combined with maximum AIL and xylan removal as well as their separation into organic and aqueous phases. High coefficients of determination for most of the resulting models showed that large portions of the variance in the responses were explained by the independent variables. Based on the numerical optimization, the highest glucose yield (alternative 1) predicted by the model was 98%, which corresponded to treatment settings at 20 min, 136 °C, 0.75% catalyst, and 16% MIBK. At these actual processing conditions, the model predicted 83% of AIL and 95% of xylan removal from the SG biomass and 76% of the xylose separated to the aqueous fraction. Alternative 2, with the lowest levels of the four processing factors (20 min, 136 °C, 0.57% catalyst, 12% MIBK) predicted relatively high glucose yield (91%); however, only catalyst and MIBK concentration were reduced in comparison with the first alternative. Also, these conditions predicted slightly lower values of AIL extraction (80%), xylan removal (93%), and xylose separated to the aqueous fraction (72%). Following alternative 3, the highest glucose yield (96%), together with maximizing delignification (87%) and xylan removal (98%), was predicted with the following treatment conditions: 40 min, 139 °C, 0.75% catalyst and 8% MIBK. These conditions predicted 79% of xylose transferred to the aqueous fraction. Finally, for alternative 4, the highest glucose yield (94%) together with maximizing delignification (92%) and xylan removal (97%) was predicted with the following treatment conditions: 40 min, 136 °C, 0.75% catalyst and 28% MIBK. These conditions predicted that 53% of the xylose separated to the aqueous fraction and dried lignin fraction with more than 100% of initial AIL content in the raw SG. Low xylose separation to the aqueous fraction (in the case of alternative 4), is a result of high MIBK content in the solvent mixture used to produce high delignification and lignin separation to the organic phase. The xylose and lignin separation to the aqueous and organic fractions, respectively, is determined by MIBK content in the solvent mixture. If the highest xylose separation to the aqueous fraction was the main goal for the study, then alternative 3 is a better option. Therefore, alternative 3 seems to be the most attractive (commercially) for selective fractionation of lignocellulosic biomass components. The effect of dilute acid treatment alone on the biomass delignification and its enzymatic digestibility was evaluated for comparative reason. Treatment at 140 °C, 0.75% catalyst, 40 min resulted in 54% glucose yield, 85% xylan removal and 17% delignification which were significantly lower when compared to similar treatment conditions with solvent mixture studied in this paper. In general, all of the alternatives examined resulted in efficient component separation; however, selection of the most advantageous processing conditions is strongly dependent upon the overall economics of the process and the fate of all the output streams. Therefore, specific applications for hemicellulose and lignin fractions would have to be proposed. Also, economic analysis is needed to determine which conditions are most profitable.

3. Conclusion

Clean fractionation pretreatment applied to switchgrass resulted in high lignin and xylan removal, leaving solid cellulose pulp. High content of glucan in the pulps, and high glucose yields obtained from enzymatic hydrolysis of the pulps, confirmed high potential of the CF pretreatment. Additionally, relatively high separation of xylose and lignin fractions suggests applicability of the CF technology in the biorefinery concept. Variations of temperature and catalyst concentration were found to be most influential on glucose yield response. MIBK content in the solvent mixture was responsible for AIL and xylose distribution between phases. Time had the least effect on the responses.

Acknowledgements

This work was supported by funding from the South Dakota State University Agricultural Experiment Station, South Dakota 2010 Centers of Excellence and Sun Grant Initiative Dept. of Transportation.

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