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Integration of extrusion and clean fractionation processes as a pre-treatment technology for prairie cordgrass

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HIGHLIGHTS

- Prairie cordgrass (PCG) was extruded at predetermined optimized conditions.
- Clean fractionation (CF) processing was performed on extruded prairie cordgrass.
- Pulps were enzymatically hydrolyzed and glucose yields were measured.
- Optimal conditions resulted in 92% glucose yield, 87% lignin and 95% xylan removal.
- Pre-extrusion improved fractionation effectiveness and glucose yield.

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ABSTRACT

Prairie cordgrass (PCG) was pretreated by sequential extrusion and clean fractionation (CF) processing. Following CF, PCG was fractionated into cellulose, hemicellulose and lignin-rich fractions. Cellulose pulp was then enzymatically hydrolyzed, producing glucose. The main purpose of this study was to produce the highest glucose yield as possible. The effects of time, temperature, catalyst concentration and solvent mixture composition on the fractionation were tested. Different proportions of methyl isobutyl ketone (MIBK), ethanol and water with sulfuric acid as a catalyst were evaluated. Optimal conditions for sequential extrusion and clean fractionation (39 min, 129 °C, 0.69% catalyst, and 28% MIBK) resulted in higher glucose yield (92%), and more lignin (87%) and xylan (95%) removal than for clean fractionation alone. Pairwise comparison of raw PCG with extruded PCG clean fractionation revealed no difference in glucose yields, but xylan and AIL removal were higher in the case of clean fractionation of the pre-extruded PCG.

1. Introduction

Lignocellulose has been gaining interest of researchers due to its tremendous potential for replacing petroleum in production of energy, chemicals, polymers and pharmaceuticals. Lignocellulosic materials which attract the most attention include softwoods, herbaceous energy crops, and agricultural residues (González-García et al., 2010). Prairie cordgrass is a warm-season prairie grass, widely used in ethanol production research (Cybulska et al., 2009; Karunanithy and Muthukumarappan, 2011b), mainly because it does not compete with food or feed production (due to its coarseness), is relatively high in cellulose, is tolerant to difficult growing conditions, and it is easily available since its growing rate is high (up to 9696 kg DM (dry matter) ha^{-1} can be produced in eastern South Dakota) (Boe and Lee, 2007; Lee et al., 2007). High bulk density of prairie cordgrass (195 kg/m^3) (Karunanithy and Muthukumarappan, 2011a) can improve storability, reduce transportation costs, and ease the handling.

The high potential of lignocellulosic biomass for value added processing is associated with its complex structure, which allows generation of various bio-based products. However, due to its complex nature, lignocellulosic biomass is resistant to processing and requires an efficient pretreatment method.

Extrusion is a physical pretreatment method which utilizes elevated temperature as well as shear stresses. Providing continuous heating, mixing and shearing of the biomass, extrusion is a complex pretreatment method that results in physical and chemical changes in the lignocellulose structure (Karunanithy and Muthukumarappan, 2010). This treatment has an advantage over other physical treatments that utilize high temperatures and pressures (e.g. hydrothermal treatment, steam explosion) by using relatively low temperatures, and therefore preventing by-product

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formation. The most common and bothersome by-products (that can lead to inhibition of the fermentation process) are furfural, 5-hydroxymethyl-2-furfuraldehyde (HMF) and acetic acid (Klinke et al., 2004), which are all formed at temperatures over 180 °C. Cellulose digestibility has been found to increase after extrusion when herbaceous materials were used as feedstock. Pretreatment optimization study performed on prairie cordgrass using a single screw extruder revealed maximum glucose, xylose, and combined sugar yields of 48.3%, 77.8%, and 56.9%, respectively, at the optimized conditions: barrel temperature 90 °C, screw speed 65 rpm, moisture content of 20%, and particle size of 8 mm (Karunanithy and Muthukumarappan, 2011b). Sugar recovery could be improved when combining with other pretreatments. Integration of extrusion with other processes was found to produce more digestible cellulose (Karunanithy and Muthukumarappan, 2011b; Lee et al., 2010). In this study extrusion was integrated with clean fractionation organosolv pretreatment.

Organosolv treatment is a promising biomass processing method that creates an opportunity for implementing the biorefinery concept (Wyman, 1996; Zhao et al., 2009) through biomass fractionation. The principle of this process is based on different affinities of the lignocellulosic components towards different solvents. Generally, organic solvents (alcohol, organic acid, ketone or ester) are used to dissolve the lignin fraction, while cellulose remains in the solid (Zhao et al., 2009). Hemicellulose can be extracted to aqueous phase if water is added to the process. There are many alternatives of the organosolv treatment, using different solvents or mixture of the solvents. These include ALCELL®, Lignol (ethanol as lignin solvent), Acetosolv (acetic acid as lignin solvent), Organocell (methanol as lignin solvent) (Young, 1998), or clean fractionation (MIBK and ethanol as lignin solvents) (Black et al., 1998). Clean fractionation was developed at the National Renewable Energy Laboratory (NREL) and has been found to be an efficient fractionation method when applied to prairie cordgrass, producing high lignin recoveries (87%) and glucose recoveries (84%), at 39 min, 154 °C, 0.69% catalyst, and 9% MIBK conditions (Black et al., 1998; Brudecki et al., 2012).

Since the ideal pretreatment method (applicable to all biomass types, environmentally friendly, having low cost of operation) is yet to be found, many researchers have attempted to integrate two or more pretreatment methods to lower the severity, improve efficiency and selectivity of the processes (Chandra et al., 2007; Wyman, 1996).

Organosolv lignin obtained from the CF process has lower molecular weight and no sulfur in compared with other industrial lignin production processes (Cybulska et al., 2012b). Therefore, organosolv lignin could be an attractive source of low-molecular weight phenols or aromatics. Screening for promising candidates from biorefinery lignin resulted in the following potential utilizations: power, green fuels, syngas (combustion, gasification, pyrolysis, and hydro-liquefaction), macromolecules (carbon fibers, polymer modifiers resins, and adhesives binders) and aromatic chemicals (benzene-toluene-xylene (BTX) chemicals, monomeric lignin molecules, low molecular weight byproducts, fermentation products) (Bozell et al., 2007). In recent study performed using lignin extracted by modified clean fractionation (using ethyl acetate, ethanol and water solvent mixture) from prairie cordgrass, switchgrass and corn stover, revealed a high potential for vanillin production by nitrobenzene oxidation. High yields of the vanillin were observed in all lignin types (approx. 40–60% w/w, calculated per initial lignin input weight), 48% w/w was obtained in case of PCG lignin (Cybulska et al., 2012b).

In this research, a possibility of an integrated process composed of extrusion and clean fractionation was explored. Other study focused on extrusion pretreatment optimization did not produce maximum sugar yields (Karunanithy and Muthukumarappan, 2011b). Extruded PCG was more compacted than raw material; therefore extrusion can reduce feedstock transportation cost. The integration of extrusion and clean fractionation processes was hypothesized to result in more efficient biomass component fractionation, produce higher enzymatic hydrolysis glucose yield or reduce the severity of clean fractionation process conditions, while still obtaining a high fractionation and enzymatic hydrolysis glucose yield.

The objective of this study was to optimize processing parameters of pre-extruded PCG using clean fractionation, including time, temperature, catalyst concentration, and organic solvent mixture composition. The fractionation process effectiveness was evaluated based on the glucose yield, optimization of which was the main goal of this study, while the secondary priority was maximizing the amount of extracted lignin and hemicellulose.

2. Methods

2.1. Sample preparation and extrusion

PCG biomass, with a composition of 37.43 ± 0.59% glucose, 15.48 ± 0.25% xylose, 2.83 ± 0.13% arabinose, 1.40 ± 0.32% galactose, 0.50 ± 0.11% mannose, 16.40 ± 0.67% acid insoluble lignin, and 4.84 ± 0.08% ash, calculated per dry matter (Brudecki et al., 2012), was obtained from a local farm near Brookings, South Dakota. PCG was air dried and ground in a hammer mill (Speedy King, Winona Attrition Mill Co, MN, USA) using an 8 mm sieve. The moisture content of the ground PCG samples was determined according to NREL/TP-510-42621 (Sluiter et al., 2008a), and then was adjusted to 20% wet basis (wb) with deionized water and equilibrated overnight. Next, the moisture–adjusted PCG was subjected to extrusion in a single screw extruder (Brabender Plasticorder Extruder, Model PL2000, Hackensack, NJ). The extruder's screw compression ratio was 3:1 and a barrel length-to-screw diameter (L/D) was 20:1. Predetermined optimized conditions were used for extrusion as follows: 90 °C barrel temperature, 65 rpm screw speed, 20% (wb) moisture content, and 8 mm particle size (Karunanithy and Muthukumarappan, 2011b). Extruded material

Table 1
Experimental design factors and corresponding values.

Coded factors	Factor 1	Factor 2	Factor 3	Solvent composition Factor 4		
	Time (min)	Temperature (°C)	Catalyst - H_2SO_4 (% w/w of solvent)	MIBK (% w/w)	Water (% w/w)	Ethanol (% w/w)
$-\alpha$	16	104	0.12	3	71	26
−1	20	110	0.20	7	63	30
0	30	125	0.39	16	50	34
+1	40	140	0.57	25	40	35
$+\alpha$	44	146	0.65	29	36	35
Additional runs	30	160	0.80	44	24	32

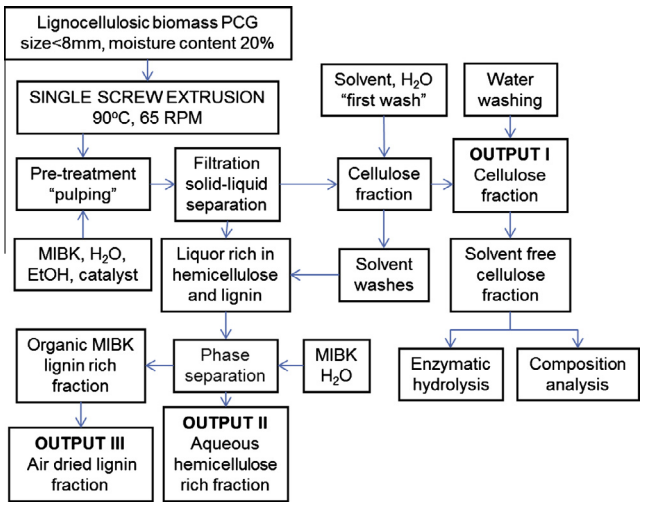

Fig. 1. Simplified flowchart of lab-scale integrated extrusion and clean fractionation (CF) process.

was collected from the die of the extruder, placed in a storage container, weighed, and kept frozen until subsequent clean fractionation treatment.

2.2. Clean fractionation (CF)

Extrusion pretreatment was followed by clean fractionation. Clean fractionation separates a lignocellulosic feedstock into individual components (i.e., cellulose, hemicellulose, and lignin) using organic solvent-aqueous mixtures containing methyl isobutyl ketone (MIBK), ethanol, and water in different proportions (Table 1). The simplified flowchart of the laboratory-scale CF process integrated with prior extrusion is presented in Fig. 1. The clean fractionation digestion equipment included stainless steel reactors and an aluminum heating block with six pockets. The heating block temperature was controlled by auto-tune temperature controllers (CN9121A, Omega). The batch reactors (capacity ~250 mL) were sealed with a screw cap utilizing a Teflon ring as a seal. In order to monitor processing conditions inside the reactors, a thermocouple and a pressure gauge were attached to the cap of each reactor. The temperature profile in each reactor was monitored by Lab View 8.2 software utilizing a data acquisition device (OMB-DAQ-56, Omega).

For each experiment 7 g DM of extruded PCG and 100 g of organic solvent mixture with catalyst were loaded and sealed in the reactors. Filled and sealed reactors were placed in the heating block and treated at different conditions, according to the experimental design presented in Table 2. Heating time was consistent throughout all the experiments between 30 and 35 min. Time zero was taken when the system reached the preset desired temperature. After the pulping process, the reactors were cooled to ambient temperature in a water bath. Digested and cooled slurry was removed from each rector and filtered through a polypropylene cloth to separate the pulp from the liquor. Water (25 mL) was used to wash the reactor and to perform the "first wash" of the pulp to remove residual solvent (Fig. 1). Liquor and "first wash" were collected and placed in a separatory funnel. "First wash" water, when added to the liquor, induced the phase separation into organic and aqueous. The pulp, which was rich in cellulose, was washed with water (around 2 L) in order to remove solvent residues. Washed pulp was collected, weighed, and kept frozen for subsequent analysis and enzymatic hydrolysis. The liquor was kept in the separatory funnel for at least an hour in order to allow complete phase separation. The two phases were drained to obtain a bottom aqueous fraction rich in hemicellulose components (output II) and the top organic fraction (output III), which was rich in lignin. The organic fraction was dried to remove the solvent, and the solid air-dried fraction rich in lignin was weighed.

Table 2
Central composite experimental design matrix.

Run	Actual				Coded			
	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)
1	20	140	0.20	25	−1.000	1.000	−1.000	1.000
2[a]	30	125	0.39	16	0.000	0.000	0.000	0.000
3	40	140	0.57	7	1.000	1.000	1.000	−1.000
4	16	125	0.39	16	−1.414	0.000	0.000	0.000
5	30	125	0.65	16	0.000	0.000	1.414	0.000
6	40	110	0.20	25	1.000	−1.000	−1.000	1.000
7[a]	30	125	0.39	16	0.000	0.000	0.000	0.000
8	44	125	0.39	16	1.414	0.000	0.000	0.000
9	40	140	0.20	7	1.000	1.000	−1.000	−1.000
10	20	110	0.57	7	−1.000	−1.000	1.000	−1.000
11	30	125	0.12	16	0.000	0.000	−1.414	0.000
12	20	110	0.20	7	−1.000	−1.000	−1.000	−1.000
13	30	104	0.39	16	0.000	−1.414	0.000	0.000
14	30	146	0.39	16	0.000	1.414	0.000	0.000
15	30	125	0.39	29	0.000	0.000	0.000	1.414
16[a]	30	125	0.39	16	0.000	0.000	0.000	0.000
17	40	110	0.57	25	1.000	−1.000	1.000	1.000
18	30	125	0.39	3	0.000	0.000	0.000	−1.414
19[a]	30	125	0.39	16	0.000	0.000	0.000	0.000
20[a]	30	125	0.39	16	0.000	0.000	0.000	0.000
21	20	140	0.57	25	−1.000	1.000	1.000	1.000
Additional runs								
22	30	160	0.80	44	−1.000	2.333	2.243	3.111
23	30	160	0.57	7	−1.000	2.333	1.000	−1.000
24	30	140	0.80	7	−1.000	1.000	2.243	−1.000
25	30	140	0.57	44	−1.000	1.000	1.000	3.111

[a] Replicates of center points in experimental design.

2.3. Experimental design for clean fractionation pretreatment optimization

The clean fractionation processing factors and corresponding treatment factor levels are presented in Table 1. The formal experimental design with treatment conditions is presented in Table 2. Response surface methodology (RSM) was used to design the experiment. A central composite design (CCD) was chosen to explore the effect of four factors: temperature (104–146 °C), digestion time (16–44 min), MIBK content in the organic solvent mixture (3–29% of MIBK) and the quantity of the catalyst (0.12–0.65% H_2SO_4) on responses such as glucose yield and delignification extent. MIBK contents and acid concentrations were selected based on a preliminary study. The experimental region included 8 factorial design points, eight axial points ($\alpha = 1.41$), and 5 replicates of the center point. Based on our previous clean fractionation study on raw PCG (Brudecki et al., 2012), four additional points were included in this design to expand the operability region, in order to find an optimum of the glucose yield response. The coded and actual values of the four independent variables are presented in Table 2. All clean fractionation treatments were performed in duplicate. Ternary mixture phase diagram was used to select the proportions of the components (MIBK/EtOH/H_2O) for this study in the same way as in previous work (Brudecki et al., 2012). Solvent ratios were chosen such that mixture remained as a single liquid phase, departed by 5% from phase transition line into single phase region direction. Sulfuric acid (H_2SO_4) was used as a catalyst. MIBK, ethanol and sulfuric acid (72%) used for preparation of the solvent mixture were purchased from Fisher Scientific.

2.4. Mass balance and fraction composition

Cellulose-, hemicellulose-, and lignin-rich fractions were weighed to perform mass balance. The total solid (TS) content was measured in the cellulose and hemicellulose fractions to determine pulp yield and solids distribution between the fractions. The pulp yield was determined by taking the ratio between the dry weight of the obtained cellulose fraction and the dry weight of the extruded PCG input (as a percentage). Compositional analyses of the dried, ground cellulose samples (output I, Fig. 1) were performed by acid hydrolysis according to NREL procedure NREL/TP-510-42618 (Sluiter et al., 2008b). The composition of the aqueous fraction (output II, Figure I), which was rich in hemicellulose components, was analyzed by injection of 10 μL of the sample into an HPLC system (Agilent Technologies System 1200 with Biorad Aminex 87H Column) with RID detector, 0.005 M H_2SO_4 mobile phase at a flow rate of 0.6 mL/min at 65 °C, as designated in NREL/TP-510-42623 (Sluiter et al., 2008b). One analysis was performed for each duplicate run, thus $n = 2$ for each treatment.

2.5. Enzymatic hydrolysis of cellulose fraction

Following clean fractionation treatment of extruded PCG, cellulose fraction samples were enzymatically hydrolyzed in order to evaluate the effectiveness of the pre-treatment. Enzymatic hydrolysis was carried out according to NREL protocol NREL/TP-510-42629 (Selig et al., 2008). Cellulase and β-glucosidase enzymes were provided by Novozymes. The dosage of the cellulase (NS50013, activity 70 FPU/g, FPU = Filter Paper Units) enzyme was chosen to be 15 FPU/g DM, whereas β-glucosidase (NS50010, activity 250 CBU/g DM, CBU = Cellobiase units) dosage was 60 CBU/g DM. Hydrolysis was conducted in an incubated shaker (Incubated Tabletop Orbital Shaker Model 420, Fisher Thermo Scientific) for 72 h at 50 °C and 150 rpm. All hydrolysis runs were performed in duplicate. Samples of hydrolysates were filtered through a 0.2 μm nylon syringe filter and the concentrations of sugars, organic acids, and by-products were measured using an HPLC system (Agilent Technologies System 1200 with BIO-RAD Aminex 87H Column) with RID detector, 0.005 M H_2SO_4 mobile phase at a flow rate of 0.6 mL/min at 65 °C, and a sample injection volume of 20 μL, according to NREL/TP-51042623 (Sluiter et al., 2008b).

The chemical used in the analyses of the enzymatic hydrolysates and aqueous fractions by high performance liquid chromatography (HPLC) was sulfuric acid (72%) (Fisher Scientific, Ricca Chemical). The chromatography standards used were D-(+)- cellobiose (Fluka), D-(+)- glucose (Sigma), D-(+)- xylose (Acros Organics), D-(−)- arabinose (Acros Organics), D-(+)- galactose (Acros Organics), D-(+)- mannose (Acros Organics) acetic acid (Sigma Aldrich), 2-furfuraldehyde (Fluka) and 5-hydroxymethyl-2-furfuraldehyde (HMF, Sigma Aldrich Fine Chemicals).

2.6. Statistical analysis

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. Statistical validation was performed by a one-way ANOVA test with 95% confidence (p-value <0.05). Backward elimination of model terms was employed, and only the terms with statistically significant coefficients were included in the 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. Lack of fit and coefficient 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 were presented as well. In general, predicted and adjusted R^2 values were assessed to be within 0.2 of each other.

3. Results and discussion

3.1. Mass balance

Mass balances of the CF process for the extruded PCG are presented in Table 3. The pulp yields of the pretreated material ranged between 26% and 78%. The final regression model included quadratic terms for temperature and acid concentration, and was the best fit for pulp yield (Table 4). Based on a perturbation plot, catalyst and temperature affected pulp yield response the most, therefore the plot showing pulp yield as a function of catalyst and temperature is presented in Fig. 2a. The paired t-test of differences between pulp yields produced from raw and pre-extruded PCG showed significant difference at the 95% confidence level in favor of the raw PCG. This indicates that more components were leached during clean fractionation of extruded material, which improves the component separation.

The air-dried lignin fraction varied between 0.18 and 2.21 g. The highest results corresponded to the highest amount of MIBK used in the pulping solvent mixture (run 22 and 25). Run 21 (25% MIBK) and run 5 (0.65% catalyst) also resulted in relatively high production of air-dried lignin fraction. The lowest dried lignin fraction yield (0.18 g) was observed for run 11 with the lowest concentration of the catalyst (0.12%). The best fit regression model equation and R-squared values are presented in Table 4. Time was determined to be a non-significant factor for this response. This is in agreement with previous work on raw PCG clean fractionation (Brudecki et al., 2012) and similar work on woody feedstock, where influence of time variation on the effectiveness of lignin removal was low (Bozell et al., 2011). The response surface of air-dried lignin fraction as a function of MIBK and catalyst concentration is

Table 3
Mass balances of CF treatments.

Run	Extruded PCG biomass DM[b] input (g)	Output I DM[b] output in cellulose fraction (g)	Pulp yield (%)	Output II DM[c] output in hemicellulose fraction (g)	Output III Dried lignin fraction (g)
1	7.00	4.60	65.67	1.02	0.49
2[a]	7.00	3.49	49.86	1.85	0.84
3	7.00	2.57	36.76	2.62	0.76
4	7.00	3.80	54.33	1.64	0.65
5	7.00	2.79	39.90	2.42	1.21
6	7.00	5.26	75.14	0.69	0.31
7[a]	7.00	3.74	53.44	1.64	0.82
8	7.00	3.62	51.77	1.90	0.82
9	7.00	4.28	61.14	2.03	0.43
10	7.00	4.24	60.61	1.65	0.39
11	7.00	5.31	75.81	0.79	0.18
12	7.00	5.48	78.23	0.81	0.24
13	7.00	4.91	70.21	1.11	0.27
14	7.00	2.79	39.91	2.94	0.80
15	7.00	3.38	48.26	2.06	0.73
16[a]	7.00	3.63	51.81	1.80	0.72
17	7.00	3.50	49.94	1.77	0.83
18	7.00	3.92	55.99	2.02	0.27
19[a]	7.00	3.87	55.29	2.05	0.67
20[a]	7.00	3.63	51.81	2.17	0.56
21	7.00	2.62	37.50	2.03	1.50
22	7.00	1.80	25.74	1.46	2.21
23	7.00	2.19	31.30	2.55	0.87
24	7.00	2.45	34.97	2.58	0.79
25	7.00	2.23	31.82	1.56	1.81

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

presented in Fig. 2b. Most of the runs using extruded PCG produced higher dried lignin fraction yields than did the raw PCG investigated in previous work (Brudecki et al., 2012). The paired *t*-test of differences between air dried lignin produced from raw and pre-extruded PCG showed significant difference at the 95% confidence level in favor of the pre-extruded PCG. This indicates that more air dried lignin fraction was produced when pre-extruded PCG was clean fractionated.

3.2. Fraction composition

Composition analysis of the pulp included glucan, xylan and AIL and is presented in Table 5. Glucan recovery in the pulp ranged between 64% and 93%, which confirms that most of the cellulose is insoluble in the solvent mixture. These results are comparable with a study performed on raw PCG (Brudecki et al., 2012) and on hardwoods such as oak and poplar (Bozell et al., 2011). A two factorial interaction regression model for glucan recovery in pulp yield is presented in Table 4. Linear terms of all factors were included in the model; however, catalyst and temperature were found to be the most significant factors, increase of each having a negative effect on the glucan remaining in the pulp (Fig. 2c).

Xylan remaining in the pulp spanned from 0% to 74%. Generally the same trend of xylan recovery was observed in the case of both extruded and raw material. However, most of the runs in this study (using extruded PCG) resulted in lower xylan recovery in pulp (higher xylan removal) when compared to the raw PCG examined in previous work (Brudecki et al., 2012). The paired *t*-test of differences between xylan removal from raw and extruded PCG showed a significant difference at the 95% confidence level in favor of extruded PCG. This indicates that pre-extrusion enhanced xylan removal, and in turn, the fractionation of lignocellulosic biomass components.

The lowest xylan recovery in the pulp occurred in treatments with the lowest pulp yield (runs 3, 5, 14, 21–25) (Table 5). These runs were performed at high levels of catalyst and/or temperature. Interestingly, for the same runs, enzymatic hydrolysis of the pulps resulted in higher xylose concentration than obtained by acid hydrolysis. It can be presumed that for these runs enzymatic hydrolysis was more effective than acid hydrolysis. On the contrary, runs carried out at low levels of catalyst and/or temperature resulted in high xylose recovery (around 70%) in the pulp (runs 6, 11, 12, 13). These results indicate that CF treatment under more severe conditions resulted in most of the hemicellulose being leached from the biomass and recovered in the aqueous liquor as xylooligosaccharides and xylose. Romani et al. (2011) reported similar results when working with Eucalyptus globulus wood fractionation; however, water autohydrolysis and subsequent ethanol solvent delignification was applied in that study (Romani et al., 2011). The final regression model for xylan remaining in the pulp

Table 4
Resulting regression models (AIL denotes acid insoluble lignin).

Regression model final equations in terms of actual factors, where A = time, B = temperature, C = catalyst, D = MIBK	R^2	Adjusted R^2	Predicted R^2
Pulp yield (%) = +259.33641 + 1.98188 * A − 2.50648 * B − 70.22104 * C − 0.91086 * D − 0.013493 * A * B − 0.022392 * A * D − 0.43447 * B * C + 0.012503 * B * D − 0.87515 * C * D + 8.78455E−003 * B^2 + 93.59497 * C^2	0.9930	0.9872	0.9691
Air dried lignin fraction (g) = −5.39421 + 0.074103 * B + 1.69380 * C − 3.93990E−003 * D + 0.053634 * C * D − 2.48491E−004 * B^2 − 1.39769 * C^2	0.9432	0.9242	0.8624
Glucan remaining in the pulp (%) = +87.28620 + 0.30630 * A − 0.018160 * B + 67.28874 * C − 0.11324 * D − 0.85234 * A * C − 0.47351 * B * C	0.8570	0.8093	0.7578
Xylan remaining in the pulp (%) = +493.69823 − 5.46319 * B − 53.61997 * C − 3.27658 * D − 1.18154 * B * C + 0.029864 * B * D − 1.67622 * C * D + 0.017993 * B^2 + 161.11959 * C^2	0.9656	0.9484	0.8536
Logit(AIL extracted from extruded PCG (%)) = Ln[(AIL extracted from extruded PCG (%) − 20.00)/(100.00 − AIL extracted from extruded PCG (%))] = −17.77949 − 0.15124 * A + 0.19638 * B + 7.03243 * C + 0.14772 * D + 1.14487E−003 * A * B + 0.044718 * A * C + 0.027848 * B * C − 8.45922E−004 * B * D − 7.04464E−004 * B^2 − 7.66581 * C^2	0.9951	0.9916	0.9841
Xylose released to aqueous fraction (%) = +486.15780 – 5.43321 * A − 6.12926 * B − 306.42037 * C + 1.28672 * D + 0.044908 * A * B + 2.34697 * B * C + 0.018003 * B^2 + 75.06131 * C^2 − 0.026917 * D^2	0.9671	0.9459	0.7953
Glucose released to aqueous fraction (%) = +23.75079 − 0.058523 * A − 0.34267 * B + 2.29282 * C + 0.55643 * D − 4.88904E−003 * B * D + 1.85799E−003 * B^2	0.8299	0.7732	0.6084
1/Sqrt(Enzymatic hydrolysis glucose yield (%)) = +0.70499 + 6.04928E−004 * A − 6.97857E−003 * B − 0.26954 * C + 1.20483E−003 * D − 5.37606E−005 * A * D + 2.35966E−005 * B^2 + 0.19648 * C^2	0.9627	0.9473	0.8910
Logit(Enzymatic hydrolysis efficiency (%)) = Ln[(Enzymatic hydr. efficiency (%) − 20.00)/(100.00 − Enzymatic hydr. efficiency (%))] = −1.33249 − 0.37032 * A − 7.88260E−003 * B − 7.53315 * C + 0.46934 * D + 2.77219E−003 * A * B + 0.11734 * B * C − 3.92099E−003 * B * D + 2.23161E−003 * D^2	0.9788	0.9682	0.7663

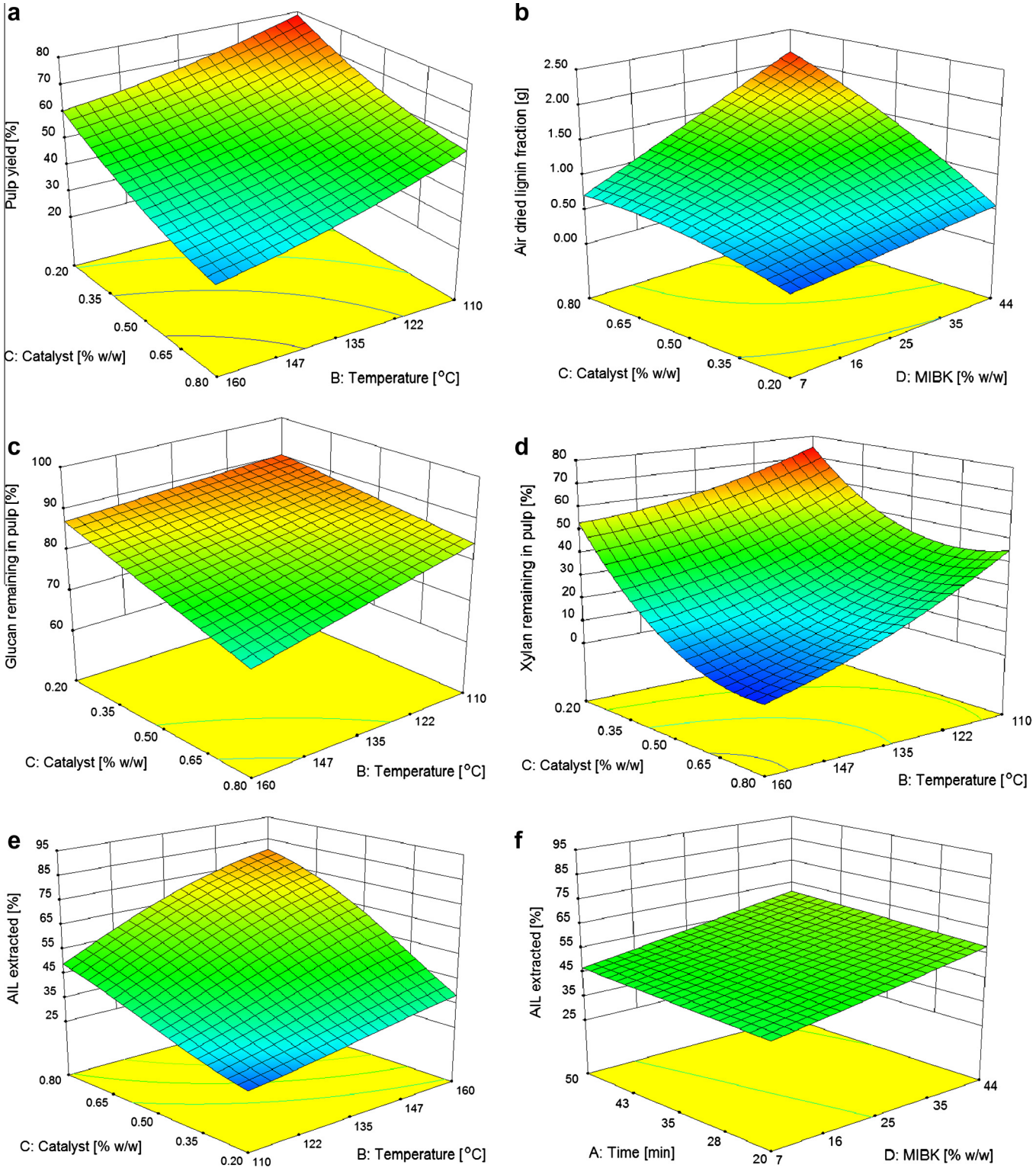

Fig. 2. Response surface plots of evaluated response variables (a) pulp yield, (b) air dried lignin fraction, (c) glucan remaining in cellulose fraction, (d) xylan remaining in cellulose fraction and (e and f) AIL (acid insoluble lignin) extracted from biomass vs. significant factors.

is presented in Table 4. Quadratic terms for temperature and catalyst were included in the model, while time was found to be an insignificant factor for this response. The effects of temperature and catalyst on xylan recovery in the pulp are presented in Fig. 2d.

The acid insoluble lignin (also called Klason lignin) content in the pulp ranged between 12% and 76% (Table 5). The lowest amount of Klason lignin, which also reflects the high extent of delignification, was observed in runs performed at high levels of catalyst and/or temperature (runs 3, 5, 14, 21–25). Low quantities of extracted AIL were correlated with low-temperature and low-catalyst (runs 6, 11, 12, 13). These runs resulted in less than 32% of AIL extraction. Similar results were observed for raw PCG clean fractionation optimization (Brudecki et al., 2012); however, more AIL lignin was extracted in the case of extruded PCG. The paired

Table 5
Composition of the pulps and aqueous fractions and enzymatic hydrolysis yields and efficiencies obtained from clean fractionation of extruded prairie cordgrass (PCG).

Run	Glucan remaining in the pulp (%)[b,e]	Xylan remaining in the pulp (%)[c,e]	AIL remaining in the pulp (%)[d,e]	Glucose released to aqueous fraction (%)[b]	Xylose released to aqueous fraction (%)[c]	Enzymatic hydrolysis xylose yield (%)	Enzymatic hydrolysis glucose yield (%)	Enzymatic hydrolysis efficiency (%)	Enzymatic hydrolysis glucose yield (%)[f]	Enzymatic hydrolysis efficiency (%)[f]
1	85.88	61.31	61.11	8.27	21.65	26.95	41.71	48.57	44.05	52.32
2[a]	83.59	33.88	43.92	8.41	24.75	27.59	52.06	62.28	58.20	69.21
3	78.46	7.28	18.43	10.88	62.85	25.17	75.95	96.80	82.15	98.72
4	82.09	41.03	50.09	7.65	22.09	28.29	49.32	60.08	46.98	53.87
5	83.09	16.43	25.27	8.47	44.52	29.86	72.74	87.54	68.94	89.93
6	92.30	70.49	72.23	7.95	20.61	14.43	34.27	37.13	32.15	34.71
7[a]	82.54	33.94	48.42	7.33	21.96	28.76	52.30	63.36	48.30	55.05
8	85.90	35.93	45.05	7.97	26.00	27.34	52.01	60.55	54.30	69.15
9	88.80	50.92	63.94	9.01	24.70	24.25	41.21	46.41	39.17	55.04
10	90.77	51.89	59.45	8.85	24.83	30.57	49.47	54.50	40.30	46.43
11	92.63	74.21	73.23	9.06	22.22	11.01	27.41	29.59	27.93	32.73
12	92.56	69.67	76.03	9.00	22.62	11.43	28.50	30.79	24.32	33.54
13	87.92	66.37	68.36	7.45	18.56	17.54	32.89	37.41	28.61	30.92
14	82.19	11.26	20.52	8.72	54.56	22.80	65.74	79.98	72.59	92.12
15	82.49	34.94	40.88	6.96	25.92	30.83	52.80	64.01	54.87	66.08
16[a]	87.72	37.60	46.89	7.89	25.67	25.27	47.57	54.23	46.75	55.95
17	83.68	33.58	42.19	7.81	24.75	33.32	57.03	68.15	45.84	53.56
18	83.73	38.33	58.74	8.93	21.93	23.63	43.97	52.51	48.42	56.68
19[a]	88.42	35.73	49.82	8.00	27.81	25.50	49.96	56.50	46.88	57.22
20[a]	82.19	35.28	47.36	6.84	24.30	29.58	52.57	63.95	51.70	60.06
21	85.60	9.14	19.87	9.95	53.25	27.82	81.80	95.57	83.86	98.41
22	63.81	0.00	11.71	6.87	23.96	0.37	63.42	99.39	62.86	92.77
23	73.56	0.00	23.58	15.21	70.63	6.22	72.52	98.59	67.51	83.79
24	77.95	5.31	24.42	13.01	69.22	18.19	77.48	99.39	66.19	83.17
25	75.39	0.00	13.05	7.63	38.47	9.75	74.56	98.90	68.75	84.66

[a] Replicates of center points in experimental design.
[b] Percent of initial glucose content in raw PCG biomass.
[c] Percent of initial xylose content in raw PCG biomass.
[d] Percent of initial AIL content in raw PCG biomass (AIL denotes acid insoluble lignin).
[e] Results obtained by acid hydrolysis.
[f] Results for clean fractionation of raw PCG (without pre-extrusion)(Brudecki et al., 2012).

t-test of differences between AIL removal from raw and extruded PCG showed a significant difference at the 95% confidence level in favor of extruded PCG. The highest delignification extent (run 22 with 88% AIL removal) was comparable to results obtained in a similar study using hardwoods as a feedstock (Bozell et al., 2011). Another study showed that clean fractionation performed on corn stover resulted in lower, but still relatively high (74%) delignification, even though the concentration of catalyst was twice as high as the concentration used in this work. Interestingly, when comparing runs 22 and 25, which were fractionated with the same composition of the solvent (44% MIBK), it can be concluded that the increase of acid content (from 0.57% to 0.80%) and temperature (from 140 °C to 160 °C) did not significantly improve the delignification (only by 1%). Also an increase of only the MIBK content from 7% to 44% did not contribute much to the extruded PCG delignification (82% vs. 87% AIL extraction, respectively), as can be observed when comparing runs 3 and 25.

Ethanol content in the five organic solvent mixtures tested was relatively constant (26–35%). This leads to the deduction that ethanol plays an important role in biomass delignification, as it has been reported in multiple publications (Zhu and Pan, 2010). However, the quantity of MIBK in the organic solvent mixture determines the ethanol distribution between the organic and liquid phases, and in turn, the separation of lignin to the organic fraction and thus the selectivity of the process. As presented in Table 3, run 3 with only 7% MIBK resulted in only 0.76 g of dried lignin fraction, whereas run 21 (25% MIBK) and run 25 (44% MIBK), generated 1.50 g and 1.81 g of dried lignin fraction respectively. The content of AIL in these fractions was 41%, 73% and 90% (as a percent of initial content of AIL in the raw PCG). Sugar analysis on these samples indicated insignificant residual content of glucose (<3%), xylose (<3%) and ash (<1%). The high purity of lignin samples suggests that it would be a useful feedstock for chemical production. In related work, where oak and aspen were clean fractionated in a semi-continuous mode, even higher purity (>95%) lignin was obtained with lower concentrations of MIBK (16%) in the solvent mixture. The regression model of AIL extracted from extruded PCG is presented in Table 4. The quadratic terms of acid and temperature factors were found significant for this model. Also these factors were found to be the most influential on the delignification of the extruded PCG, as presented in Fig. 2e–f. In related work, Klason lignin content remaining in the pulp was also found to be strongly correlated with acid concentration (Bozell et al., 2011). Therefore, lignin extraction by CF process was considered relatively high for extruded and raw PCG, when compared to recent studies on a non-catalyzed organosolv treatment of PCG using ethyl acetate as the organic solvent (Cybulska et al., 2012a).

The aqueous fraction contained monomeric and oligomeric hemicellulose originated sugars, depolymerized lignin, and other unidentified components. Since composition analysis of the dried lignin fraction revealed only trace amounts of monomeric sugars, xylose and glucose leached from biomass were assumed to be selectively transferred to the aqueous fraction. The xylose and glucose content in the aqueous fractions is presented in Table 5. Xylose content in the aqueous fractions ranged between 19% and 71%. The oligomeric hemicellulosic sugars and degradation by-products can be determined as the difference between xylose removed from the extruded PCG and monomeric xylose in the aqueous fraction.

As a result of a dominant content of xylose in the hemicellulose (more than 75%), xylose measurements indicated the extent of hemicellulose polymer fragmentation. The highest amount of xylose released to the aqueous fraction corresponded to runs 3, 14, 21, 23, 24 (53–71%). These runs were performed with high

temperature and high catalyst concentration. Interestingly, runs 22 and 25, also performed at high temperatures and catalyst concentrations, did not result in high xylose content in aqueous fraction, even though complete removal of xylan from the cellulose fractions occurred for these runs. Acid hydrolysis of aqueous fraction also did not generate significant increase of xylose concentration for this run, indicating a small content of xylooligosaccharides. Compositional analysis of the dried lignin fraction did not reveal significant content of sugars in these runs either. Therefore, it is suspected that a significant part of the xylose was decomposed or irreversibly bonded to lignin in this treatment's conditions. Similar results were observed for clean fractionation of raw PCG (Brudecki et al., 2012).

The formation of furfural and HMF is undesirable because these by-products represent a loss of fermentable sugars. Up to 4% of the initial glucose present in raw prairie cordgrass was converted to HMF and detected in the aqueous fraction, while no furfural was detected in the aqueous fraction. Similar quantities of HMF and furfural were detected in the aqueous fraction when raw prairie cordgrass was clean fractionated. Trace quantities of furfural (0.45% of the initial xylose present in raw prairie cordgrass) and HMF (0.69% of the initial glucose present in raw prairie cordgrass) were detected in the dried lignin fractions. As reported in other studies (Negro et al., 2003; Novo et al., 2011), harsh treatment conditions (mainly high temperature) can lead to the breakdown of the released sugars during the pretreatment step. Decomposition products, such as furan derivatives, can be formed. It includes furfural formed from pentose sugars and 5-hydroxymethylfurfural (HMF) generated from hexose sugar degradation. Even further degradation can occur, where HMF breaks down to form equimolecular amounts of levulinic and formic acids, while furfural can be degraded to formic acid. The furan derivatives and soluble phenolic compounds from lignin can react further to form some polymeric materials. Formation of polymeric condensation products may help to explain low total xylose recoveries in all fractions for experiments 22 and 25. Also it should be noted that measurement of Klason lignin content for run 22 resulted in 120% of the initial lignin content in the raw PCG. As reported by Chandra et al. (2007), the 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". This can result in artificially high values for Klason lignin (also called "pseudolignin") and explain missing sugars (Chandra et al., 2007; Garrote et al., 1999).

A statistically significant model was built for xylose released to the aqueous fraction, and is presented in Table 4. The quadratic terms of temperature, catalyst and MIBK concentration factors were significant at the 95% confidence level. Increase of catalyst and temperature resulted in higher xylose concentration in monomeric form in the aqueous fraction, while MIBK content also had a positive effect on this response, but to a lesser extent. Too high a concentration of MIBK resulted in a decrease of monomeric xylose in the aqueous fraction. It can be observed when comparing run 21 to run 25 that increasing MIBK from 25% to 44%, while keeping the temperature and catalyst at the same levels, resulted in 53% and 38% of xylose in the aqueous fraction, respectively. It indicates some affinity of xylose or xylose degradation products to organic fraction.

According to the clean fractionation concept, the extraction of glucose to the aqueous fraction is undesirable because it represents a loss of fermentable sugars and reduces glucose yield in the enzymatic hydrolysis. Glucose extracted to the aqueous fraction was relatively consistent throughout this study, and ranged between 7% and 9% for most of the runs. However, runs 3, 23 and 24 resulted in slightly higher glucose content in the aqueous fraction (11%, 15% and 13%, respectively). These runs were performed at severe conditions with low MIBK content in the solvent mixture. A statistically significant model was built for glucose released to the aqueous fraction and is presented in Table 4, and the quadratic term of the catalyst concentration factor was significant at the 95% confidence level.

In order to follow the biorefinery concept and to ultimately make the CF process profitable, all output streams need to be utilized. Hemicellulose and lignin fractions should be subjected to further investigation for specific chemical production. However this was beyond the scope of this work.

3.3. Enzymatic hydrolysis of cellulose fraction

The enzymatic hydrolysis glucose yield was the crucial point of this study. The glucose yield was determined as the percentage of glucose measured in the enzymatic hydrolysate divided by the initial content of glucose in the raw PCG material. Ratio of glucose measured in the enzymatic hydrolysate to glucose submitted to enzymatic hydrolysis was used to calculate the enzymatic hydrolysis efficiency. Enzymatic hydrolysis glucose yield and enzymatic hydrolysis efficiency values are presented in Table 5. Concentration of glucose in the enzymatic hydrolysates ranged between 4 and 28 mg/mL; the maximum value of this interval was higher than that found for clean fractionated raw PCG (26 mg/mL) only (e.g., no prior extrusion), (Brudecki et al., 2012). The lowest glucose concentration, and in turn the lowest glucose yield, was observed for the runs performed at mild conditions with low concentration of acid and low temperature. Runs 6, 11, 12 and 13 produced glucose concentrations around 4–5 mg/mL, which was equivalent to glucose yields between 27% and 34%. These runs also produced some of the lowest concentrations of xylose in the enzymatic hydrolysates, and resulted in the highest amounts of xylose and AIL remaining in the pulps.

As reported previously for CF of raw PCG, high amounts of lignin and xylose remaining in the cellulose fraction limit the enzymatic actions, and in turn produce low glucose yields (Brudecki et al., 2012). Similar effects were observed in this study when working on the extruded PCG. Runs 3, 5, 14, 21–25 resulted in high glucose concentrations in the enzymatic hydrolysates (17–28 mg/mL, equivalent to 63–82% glucose yields). The highest glucose yield was observed for run 21 (82%). Previous investigation on clean fractionation of raw PCG (Brudecki et al., 2012) resulted in the highest glucose yield (84%) for the same treatment conditions (run 21). Even though the clean fractionation of the raw PCG resulted in higher glucose yield than that which was extruded, the difference was not statistically significant at the 95% confidence level. High glucose yields were also observed for runs 3, 5, 14, 22–25 (63–77%), since these runs generated high glucose concentrations in the enzymatic hydrolysates. The same runs, along with run 21, showed high enzymatic hydrolysis efficiency ($\geqslant$80%). Extent of cellulose hydrolysis was higher when compared to related work on clean fractionation of corn stover (max 70%) (Ishizawa et al., 2009). No significant differences were observed between results of glucose yield produced from raw PCG (Brudecki et al., 2012) and the extruded PCG, and the paired *t*-test of difference between them showed no significant difference at the 95% confidence level. The best results for glucose yield and the hydrolysis efficiency obtained in the present study are very close to those reported in related work using ethanol organosolv pretreatment, where enzymatic conversion yields of cellulose were $\sim$85% and $\sim$97% for hybrid poplar and beetle-killed lodgepole pine, respectively (Zhao et al., 2009).

Enzymatic hydrolysis glucose yield and enzymatic hydrolysis efficiency (based on glucose) regression models are presented in Table 4. Linear terms of four processing factors were included in both models, while the quadratic terms of temperature and

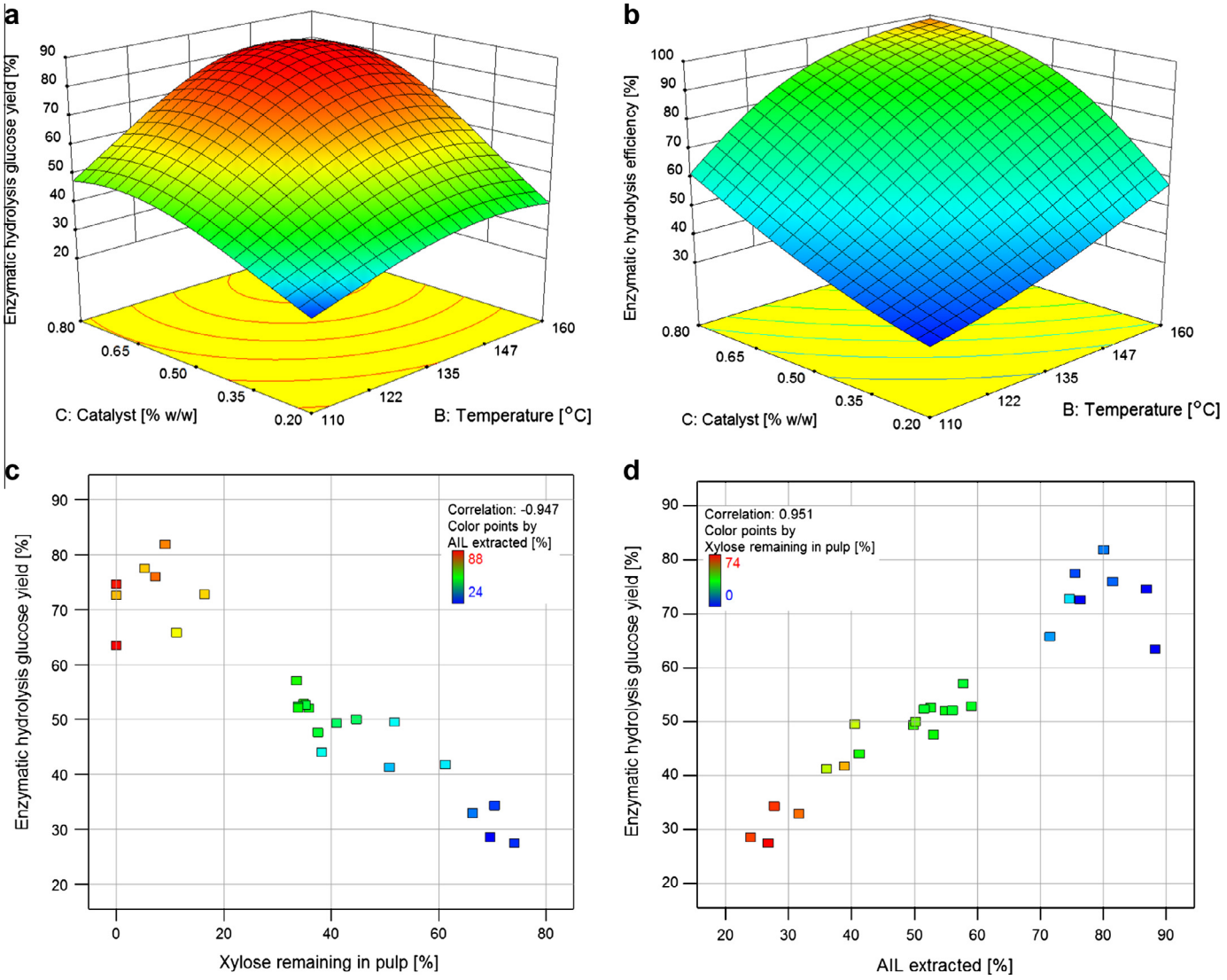

Fig. 3. Effect of catalyst (% w/w) and temperature (°C) on (a) enzymatic hydrolysis glucose yield (%) and (b) enzymatic hydrolysis efficiency (%). Correlation between enzymatic hydrolysis glucose yield and xylose remaining in cellulose (c). Correlations between glucose yield and AIL (acid insoluble lignin) extracted from biomass (d).

catalyst concentration terms were significant for the glucose yield model, and the quadratic term of MIBK content was significant for the enzymatic hydrolysis efficiency model. Enzymatic hydrolysis glucose yield and enzymatic hydrolysis efficiency responses as a function of temperature and catalyst concentration are presented in Fig. 3a–b. No significant difference was observed between results of enzymatic hydrolysis efficiencies produced from raw PCG (Brudecki et al., 2012) and the extruded PCG, and the paired *t*-test of difference between them showed no significant difference at the 95% confidence level. Enzymatic hydrolysis profile for raw and extruded clean fractionated originated pulps is presented in Fig. 4. Higher glucose production was obtained for extruded clean fractionated prairie cordgrass than for raw clean fractionated prairie cordgrass (Fig. 4).

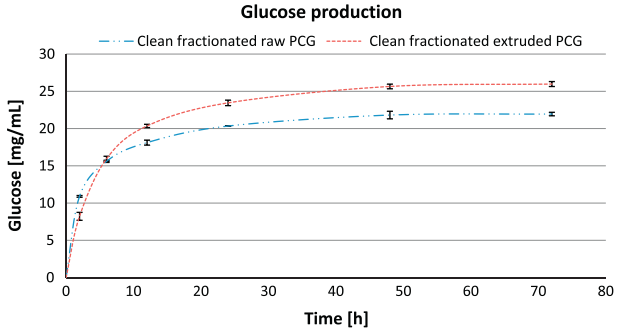

Fig. 4. Comparison of glucose production rate obtained from extruded clean fractionated prairie cordgrass and raw clean fractionated prairie cordgrass pulp.

Many researchers have reported that hemicellulose removal during pretreatment also results in significant improvement of enzymatic digestibility of the lignocellulosic substrates (Chandra et al., 2007; Mosier et al., 2005). Close associations between cellulose, hemicellulose and lignin act as physical and chemical barriers for enzymes. Hemicellulose and lignin removal result in increased pore volumes of the pre-treated material, which improves enzyme penetration and access to the glycosidic bonds (Chandra et al., 2007; Mooney et al., 1998). As presented in Fig. 3c, results obtained in this study confirm the negative correlation between xylose remaining in the cellulose fraction and glucose yield. Related research using clean fractionation as a pretreatment revealed that the enzymatic digestibility of cellulose is more significantly improved by xylan removal than by delignification. However, it was found that almost complete removal of lignin using acidified

sodium chlorite can result in a decrease in cellulose susceptibility to enzyme due to aggregation of the cellulose microfibrils (Ishizawa et al., 2009).

It has been demonstrated by various researchers that the presence of lignin affects enzymatic hydrolysis as well. Lignin can be one of the most significant obstacles for enzyme performance during the hydrolysis (Hsu, 1996; Zhu et al., 2008). Strong evidence supporting the negative influence of lignin on enzymatic hydrolysis was found in this study as well, as presented in Fig. 3d. Highly delignified samples resulted in high glucose yields, and vice versa. These results are in agreement with those obtained in previous work on clean fractionation of raw PCG (Brudecki et al., 2012). However, other studies showed that higher or even complete removal of lignin is not a requirement for high glucose yields in enzymatic hydrolysis. According to Chang and Holtzapple (2000), once the bonds between lignin and cellulose are cleaved, the hydrolysis efficiency increases, even though lignin is still present in the material (Chang and Holtzapple, 2000). Other structural properties potentially limiting enzymatic hydrolysis of cellulosic fibers are connected to cellulose's structure itself, and include degree of polymerization, crystallinity, cellulose lattice structure, structural composition, particle size, fiber dimensions, available surface area, degree of fiber swelling, pore structure and distribution (Mansfield et al., 1999; Meng et al., 2012; Mooney et al., 1998; Mosier et al., 2005).

3.4. Optimization

The purpose of this study was to optimize the process of clean fractionation performed on the extruded PCG, in order to find the highest glucose yield produced by enzymatic hydrolysis of the cellulosic pulp. Numerical methods provided several solutions for specified optimization conditions, and three different optimization alternatives were considered. High coefficients of determination for all of the models showed that the large portions of the variance in the responses were explained by the independent variables. First the highest glucose yield predicted by the model was 91.75%, which corresponded to 39 min, 129 °C, 0.69% catalyst, and 28% MIBK. At these processing conditions, the model predicted 87% of AIL and 95% of xylan removal from biomass. Application of the same processing conditions to raw PCG would result in 72% glucose yield, 72% AIL removal and 83% xylan removal, based on the previously determined models for clean fractionation of raw PCG (Brudecki et al., 2012). Secondly, lower MIBK content in solvent mixture could be used to produce still relatively high glucose yield (82%); however, higher temperature and catalyst content would have to be employed (21 min, 143 °C, 0.73% catalyst, 7% MIBK). Also, these conditions would result in lower AIL extraction (78%), but still very high xylan removal (95%). The raw PCG model predicted 76% glucose yield, 71% AIL removal, and 94% xylan removal for the same treatment conditions (Brudecki et al., 2012). Thirdly, if the goal was to find the highest glucose yield (85%), together with maximizing delignification (90%), and xylan removal (100%), the predicted treatment conditions would be 24 min, 148 °C, 0.66% catalyst, and 42% MIBK. These conditions would result in only 3% more AIL removal than initial conditions (i.e., the first optimization approach) suggested for maximum glucose yield as a result of increasing the MIBK by 14% and treatment temperature by 20 °C. At the same conditions, clean fractionation of raw PCG would result in 80% glucose yield, 88% AIL removal and 97% xylan removal (Brudecki et al., 2012). The second suggested condition seems to be the most preferable if minimal consumption of MIBK was desired. Employing optimal conditions determined for clean fractionation of raw PCG (39 min, 154 °C, 0.69% catalyst, and 9% MIBK, producing 84% glucose yield, 87% lignin removal) (Brudecki et al., 2012) to this clean fractionation of extruded PCG model resulted in lower (78%) glucose yield and higher xylan (100%) and lignin (92%) removal. Severity factor was calculated for the first alternative, leading to maximum glucose yield, according to Garrote, (1999) and was equal to log R_0 = 2.44. This value was lower in comparison with severity factor calculated for optimal conditions obtained for clean fractionation of raw prairie cord grass, where log R_0 = 3.18 (Brudecki et al., 2012). In order to select most advantageous processing conditions, specific applications for hemicellulose and lignin fractions would have to be proposed. Economic analysis would also have to be included in order to say which conditions are more profitable. If higher xylan and lignin removal could compensate for extrusion cost, clean fractionation combined with extrusion can be more beneficial.

4. Conclusion

Clean fractionation is an efficient biomass fractionation technology, resulting in an aqueous liquid phase rich in hemicellulose-derived sugars or oligomers, an organic liquid phase rich in lignin and solid cellulose pulp. Comparing optimal conditions for clean fractionation of extruded PCG to raw PCG revealed that the extruded PCG generates higher glucose yields as well as greater xylan and lignin removal. However, pairwise comparisons of the raw and extruded PCG clean fractionation responses, at the 95% confidence level, revealed no difference in glucose yields, but xylan and AIL removal was higher in case of the clean fractionation of the extruded PCG.

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References

Black, S.K., Hames, B.R., Myers, M.D., 1998. Method of separating lignocellulosic material into lignin, cellulose and dissolved sugars. D21C 3/20 ed, Midwest Research Institute, Kansas City, Mo.

Boe, A., Lee, D.K., 2007. Genetic variation for biomass production in prairie cordgrass and switchgrass. Crop Sci. 47 (3), 929–934.

Bozell, J.J., Black, S.K., Myers, M., Cahill, D., Miller, W.P., Park, S., 2011. Solvent fractionation of renewable woody feedstocks: Organosolv generation of biorefinery process streams for the production of biobased chemicals. Biomass Bioenergy 35 (10), 4197–4208.

Bozell, J.J., Holladay, J.E., Johnson, D., White J.F., 2007. Top value added chemicals from biomass, volume II: Results of screening for potential candidates from biorefinery lignin. Pacific Northwest National Laboratory Report, PNNL-16983. Pacific Northwest National Laboratory, Richland, WA.

Brudecki, G., Cybulska, I., Rosentrater, K., Julson, J., 2012. Optimization of clean fractionation processing as a pre-treatment technology for prairie cordgrass. Bioresour. Technol. 107, 494–504.

Chandra, R., Bura, R., Mabee, W., Berlin, A., Pan, X., Saddler, J., 2007. Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics? In: Olsson, L. (Ed.), Biofuels, vol. 108. Springer, Berlin/Heidelberg, pp. 67–93.

Chang, V., Holtzapple, M., 2000. Fundamental factors affecting biomass enzymatic reactivity. Appl. Biochem. Biotechnol. 84–86 (1), 5–37.

Cybulska, I., Brudecki, G., Lei, H., Julson, J., 2012a. Optimization of combined clean fractionation and hydrothermal treatment of prairie cord grass. Energy Fuels 26 (4), 2303–2309.

Cybulska, I., Brudecki, G., Rosentrater, K., Julson, J.L., Lei, H., 2012b. Comparative study of organosolv lignin extracted from prairie cordgrass, switchgrass and corn stover. Bioresour. Technol. 118, 30–36.

Cybulska, I., Lei, H., Julson, J., 2009. Hydrothermal pretreatment and enzymatic hydrolysis of prairie cord grass. Energy Fuels 24 (1), 718–727.

Garrote, G., Dominguez, H., Parajo, J.C., 1999. Hydrothermal processing of lignocellulosic materials. Holz Roh-Werks 57 (3), 191–202.

González-García, S., Moreira, M.T., Feijoo, G., 2010. Comparative environmental performance of lignocellulosic ethanol from different feedstocks. Renew. Sustain. Energy Rev. 14 (7), 2077–2085.

Hsu, T., 1996. Pretreatment of biomass. In: Handbook on Bioethanol: Production and Utilization, Taylor & Francis.

Ishizawa, C.I., Jeoh, T., Adney, W.S., Himmel, M.E., Johnson, D.K., Davis, M.F., 2009. Can delignification decrease cellulose digestibility in acid pretreated corn stover? Cellulose 16 (4), 677–686.

Karunanithy, C., Muthukumarappan, K., 2010. Effect of extruder parameters and moisture content of switchgrass, prairie cord grass on sugar recovery from enzymatic hydrolysis. Appl. Biochem. Biotechnol. 162 (6), 1785–1803.

Karunanithy, C., Muthukumarappan, K., 2011a. Optimization of alkali soaking and extrusion pretreatment of prairie cord grass for maximum sugar recovery by enzymatic hydrolysis. Biochem. Eng. J. 54 (2), 71–82.

Karunanithy, C., Muthukumarappan, K., 2011b. Optimization of extruder and prairie cord grass parameters for maximum sugar recovery through enzymatic hydrolysis. J. Biobased Mater. Bioenergy 5 (4), 520–531.

Klinke, H.B., Thomsen, A.B., Ahring, B.K., 2004. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. Appl. Microbiol. Biotechnol. 66 (1), 10–26.

Lee, D.K., Owens, V.N., Boe, A., Jeranyama, P., 2007. Composition of herbaceous biomass feedstocks. North Central Sun Grant Center Report, SGINC1-07. Plant Science Department, South Dakota State University, Brookings, SD.

Lee, S.-H., Inoue, S., Teramoto, Y., Endo, T., 2010. Enzymatic saccharification of woody biomass micro/nanofibrillated by continuous extrusion process II: Effect of hot-compressed water treatment. Bioresour. Technol. 101 (24), 9645–9649.

Mansfield, S.D., Mooney, C., Saddler, J.N., 1999. Substrate and enzyme characteristics that limit cellulose hydrolysis. Biotechnol. Prog. 15 (5), 804–816.

Meng, L.Y., Kang, S.M., Zhang, X.M., Wu, Y.Y., Xu, F., Sun, R.C., 2012. Fractional pretreatment of hybrid poplar for accelerated enzymatic hydrolysis: Characterization of cellulose-enriched fraction. Bioresour. Technol. 110, 308–313.

Mooney, C.A., Mansfield, S.D., Touhy, M.G., Saddler, J.N., 1998. The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwoods. Bioresour. Technol. 64 (2), 113–119.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M., 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol. 96 (6), 673–686.

Negro, M.J., Manzanares, P., Ballesteros, I., Oliva, J.M., Cabanas, A., Ballesteros, M., 2003. Hydrothermal pretreatment conditions to enhance ethanol production from poplar biomass. Appl. Biochem. Biotechnol. 105 (1), 87–100.

Novo, L.P., Gurgel, L.V., Marabezi, K., Curvelo, A.A., 2011. Delignification of sugarcane bagasse using glycerol-water mixtures to produce pulps for saccharification. Bioresour. Technol. 102 (21), 10040–10046.

Romani, A., Garrote, G., Lopez, F., Parajo, J.C., 2011. Eucalyptus globulus wood fractionation by autohydrolysis and organosolv delignification. Bioresour. Technol. 102 (10), 5896–5904.

Selig, M., Weiss, N., Ji, Y., 2008. Enzymatic saccharification of lignocellulosic biomass. Laboratory Analytical Procedure, NREL/TP-510-42629. National Renewable Energy Laboratory, Golden, CO.

Sluiter, A., Hames, B., Hyman, D., Payne, C., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Wolfe, J., 2008a. Determination of total solids in biomass and total dissolved solids in liquid process samples. Laboratory Analytical Procedure, NREL/TP-510-42621. National Renewable Energy Laboratory, Golden, CO.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008b. Determination of structural carbohydrates and lignin in biomass. Laboratory Analytical Procedure, NREL/TP-510-42618. National Renewable Laboratory, Golden, CO.

Wyman, C.E., 1996. Handbook on Bioethanol. Production and Utilization. Taylor & Francis.

Young, R.A., 1998. Environmentally Friendly Technologies for the Pulp and Paper Industry. John Wiley & Sons, Inc.

Zhao, X., Cheng, K., Liu, D., 2009. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Appl. Microbiol. Biotechnol. 82 (5), 815–827.

Zhu, J.Y., Pan, X.J., 2010. Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation. Bioresour. Technol. 101 (13), 4992–5002.

Zhu, L., O'Dwyer, J.P., Chang, V.S., Granda, C.B., Holtzapple, M.T., 2008. Structural features affecting biomass enzymatic digestibility. Bioresour. Technol. 99 (9), 3817–3828.