Optimization of modified clean fractionation of prairie cordgrass

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Abstract: In this study, modified clean fractionation process was optimized for prairie cordgrass, with usage of alternative organic constituent – *ethyl acetate.* Other constituents of the solvent mixture included ethanol and water. Clean fractionation solvent was used in different proportions of the constituents. Process efficiency was determined by lignin recovery, solvent composition, as well as time and temperature applied to each sequential process. Glucose yield during enzymatic hydrolysis and overall pretreatment were calculated. Optimal conditions (125℃, 37 min, with the solvent composition of ester:ethanol:water = 32.5:22.5:45) yielded a 20% lignin recovery, 38% glucose yield during enzymatic hydrolysis and 26% xylose recovery in aqueous fraction.

Keywords: clean fractionation, prairie cord grass, enzymatic hydrolysis, ethyl acetate, biomass organosolv pretreatment
DOI: 10.3965/j.ijabe.20120502.00?

Citation: Iwona Cybulska, Hanwu Lei, James Julson, Grzegorz Brudecki. Optimization of modified clean fractionation of prairie cordgrass. Int J Agric & Biol Eng, 2012; 5(2): －.

1 Introduction

Lignocellulosic ethanol is a second generation of biofuels, not competing with food production, and with an opportunity of generating a wide range of value added co-products. Herbaceous lignocellulosic feedstock includes agricultural residues (e.g. corn stover) and prairie grasses (e.g. switchgrass, big blue stem, prairie cordgrass). Prairie cord grass is an abundant and very accessible grass species growing in southeastern and southwestern part of the U.S. as well as in Canada. In contrary to other grass species, prairie cordgrass is rarely used as an animal feed due to its coarseness, which eliminated competition with feed production[1].

There are a number of pretreatment methods applied to lignocellulosic biomass under extensive research. Most of them, however, are targeted towards only enhancing carbohydrates digestibility in order to obtain the highest possible ethanol yield. Since biomass is a very abundant source, from which a wide variety of products can be generated, the best way to utilize its potential is through a biorefinery concept[2]. Fractionation processes provide approaches to fully utilize the biomass. Currently, there are several fractionation methods under development, which are proven to be effective. These include mainly organosolv treatments such as ALCELL or Lignol processes, both of which utilize ethanol as a lignin solvent. While lignin is removed to the organic fraction, cellulose is left as a solid. Lignin extracted by both of these processes was found relatively pure and highly phenolic[3-4].

Organosolv processes are generally focused on lignin removal from the lignocellulosic structure. Lignin has drawn researchers' attention as a material from which a variety of products may be derived. For example, oxidation of phenolic groups in lignin polymer can yield formation of vanillin, guaiacol, vanilic acid,

Received date: 2011-10-27 **Accepted date:** 2012-05-22
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acetovanillone, 5-carboxyvanillin, vanillil, vanillovanillone and many more[7]. Finding new high value use for lignin is basically focused on sulfur-free lignins (different from Kraft lignins), obtained from organosolv fractionation. These lignins are high purity and low molecular weight[5], are practically free of sulfur and sodium, which gives low ash content[10]. They provide greater versatility in utilization. Examples are in phenolic, epoxy, or isocyanate resins, polyurethane foams or biodispersants as well as chemicals. Wide variety of possible products is a result of its phenolic polymeric structure. Its processing does not emit irritating odors, in contrary with Kraft lignins[9].

There are different ways to employ organosolv treatments to dissolve lignin and leave cellulose as solid. These techniques differ among each other mainly by applied organic solvents, catalysts, processing time and temperature[7]. Organosolv delignification is a chemical treatment, where the basic reaction cleaves the α- and β-ether bonds within the lignin structure. Based on Hildebrand's solubility parameter (δ), a good lignin solvent should have it close to 11 cal/cm^3 (1cal = 4.1868 J)[5]. The first common method is based on alcohol pretreatment, where ethanol (δ = 12.7 cal/cm^3) and methanol (δ = 14.5 cal/cm^3) are the most popular solvents[11]. Temperatures used vary between using a catalyst (below 180℃) and not using a catalyst (185-210℃). Catalysts of choice include mineral acids, bases, magnesium, calcium or barium chloride and nitrate. This treatment has a good chance for solvent recycling. Ethanol treatment (e.g. Lignol process, since 2001, is based on wood conversion to ethanol through the application of catalytic ethanol organosolv pretreatment) can also reach high levels of glucose yield – even up to 90%-100%[5,12-13]. Another group of solvents used, however of somewhat lower interest, are organic acids (mainly acetic and formic). These solvents result in lower digestibility of cellulose than the ethanol treatment (mainly due to acetylation of cellulosic hydroxyl groups) and cause serious equipment corrosion. Ketones seem to be efficient solvents for delignification as well (e.g. acetone, with δ = 10.0 cal/cm^3, or MIBK, with δ = 8.4 cal/cm^3)[8]. These solvents are mainly used in wood treatments. Temperatures for those methods are in the range of 180-230℃, and can be lowered to around 140℃ with addition of catalyst. Ketones are immiscible with water; therefore if the treatment is to be organic-aqueous, a third compound needs to be added. This can be either low molecular weight alcohol or organic acid[5,14]. An example of an organosolv treatment using ketones is "clean fractionation", a process developed at National Renewable Energy Laboratory in the 1990s. In this process, a primary lignin solvent is methyl isobutyl ketone (MIBK), which is applied to the biomass in a one-phase mixture with ethanol and water. The process is catalyzed with sulfuric acid, to perform the treatment at temperatures near 140℃[14].

There still are a number of possible effective lignin solvents, which could be used in organosolv pretreatment. These include aldehydes, phenols, thio-compounds, dioxane, organic bases, dimethyl sulfoxide, and esters (ethyl acetate, butyl acetate)[7]. Ethyl acetate used commercially as a replacement for e.g. acetone, has δ = 9.1 cal/cm^3 and has possibility of lowering the cost and toxicity of the process[11]. Ethyl acetate used with alcohol (e.g. ethanol) or organic acid (e.g. acetic) can easily form a one phase mixture with water across a range of proportions[15]. Its solubility in water is four times higher than that of MIBK. Furthermore, it has low boiling point – 77.1℃, which could improve the solvents recovery.

Any organosolv treatment is associated with cost of chemicals and high energy demands – since high temperatures are being used. However, the process can be economically feasible when the chemicals are recovered in the most part. For example, according to the study of Garcia et al.[16], in optimized Lignol process, 98% of ethanol input and 82% of water input is being recovered, which reduces the expenses and improves the overall economy of the process.

This study modified the original clean fractionation procedure by replacing the toxic MIBK with an alternative solvent – ethyl acetate, which is less hazardous and less expensive. Also, to lower the harshness of the process, elimination of the catalyst was examined. The main purpose of the optimizations was to find possibly the mildest conditions with the highest lignin recovery and

cellulose digestibility.

2 Materials and methods

2.1 Biomass preparation

Prairie cordgrass (PCG) was harvested in Brookings, South Dakota, US. Compositional analysis of the PCG was performed via acid hydrolysis, according to Hames et al.[17], and the results are given in Table 1. Hemicellulose was composed of ~80% xylose, containing small amounts of arabinose, mannose and galactose. Therefore xylose was considered as representative main component of hemicellulose and monitored in the experiments.

Table 1 Prairie cordgrass composition

Glucose [%DM]	Xylose [%DM]	Arabinose [%DM]	Mannose [%DM]	Galactose [%DM]	Lignin [%DM]	Extractives [%DM]	Ash [%DM]
36.70 +/- 0.01	13.52 +/- 2.00	1.59 +/- 0.57	1.00 +/- 0.05	1.50 +/- 0.03	20.96 +/- 0.52	19.00 +/- 1.00	5.65 +/- 0.04

2.2 Clean fractionation (CF)

Clean fractionation was performed in pressure reactors with 10 g of biomass dry matter (DM) content in 100 g of the solvent. The reactor experimental setup was custom-made, and contained 6 pressure reactors with 250 mL capacity. Process was conducted with controlling the temperature and monitoring the pressure (aided by LabView version 8.2). Prior to the experiment, PCG was ground to pass through a 1-mm screen (Thomas-Wiley Laboratory Mill, Model 3375-E15, Thomas Scientific, USA) to ensure uniformity of the samples.

The solvent was composed of ethyl acetate, ethanol and deionized water in different proportions (ranging from 3:22.5:74.4 as the lowest ethyl acetate content and 50:35:15 as the highest ethyl acetate content). Ethyl acetate was chosen to replace MIBK in standard clean fractionation[11] especially due to its low toxicity (NFPA Health Hazard is at level 1, while for MIBK is 2). The content of ethyl acetate and ethanol in the solvent mixture used in the trials (as two of the factors being optimized) can be found in section 2.5. Ranges of these factors were based on maintaining one-phase mixture of the solvent at room temperature[18].

The reaction temperature was examined in the range from 100 to 150℃ with time between 3 and 30 min (starting from the water b.p. and ending just over the NREL's optimized temperature)[14]. The reactors were placed in an insulated heating block, and preheated until reaching the desired temperature (about 40 min). After the process time was over, reactors were cooled in the cold water bath about for 20 min. The experimental plan for the time and temperature factors is shown in the section of Response surface analysis. The trials were carried out without any external catalyst added.

After the reaction, the cellulose rich fraction (solid) was filtrated, and extensively washed with water. The liquid fraction was separated into organic (containing lignin) and aqueous phase containing hemicellulose, by-products and residual organics). In order to achieve phase separation, as well as to avoid the deposition of hydrophobic lignin on cellulose fibers, it was necessary to wash the solids with water and organic solvent (20 g ethyl acetate and 80 g water), prior to filtration. The organic fraction was then evaporated, leaving lignin containing solid residual. The aqueous fraction was analyzed for the sugars and by-products, while the solid fraction was enzymatically hydrolyzed.

2.3 Hydrolysis

Hydrolysis was performed according to NREL protocol[19]. The hydrolysis was conducted in 100 mL mixture and monitored by collecting 1.5 mL samples after 0, 3, 6, 12, 24, 34, 48 and 72 h. Hydrolysis was performed using cellulase (Novozymes, NS50013) and β–glucosidase (Novozymes, NS50010), added in amounts 15 FPU/gDM (Filter Paper Units per gram of dry matter) and 60 CBU/gDM (Cellobiase Unit per gram of dry matter) respectively. Biomass was placed in the 250 mL flasks in amount adjusted to achieve 3 g of DM along with 0.1 M citric buffer with pH 4.8 (50 mL). DI water was added to bring total volume to 100 mL. Sodium azide was added in order to maintain sterile conditions and avoid bacterial contamination.

Hydrolysis was performed in duplicates. Concentrations of sugars as well as by-products were measured on High Performance Liquid Chromatography (Agilent HPLC 1200 Series) instrument and samples were prepared

according to LAP 015[20] and LAP 013[21]. The column used for sugar and by-products analysis was Aminex HPX-87H with the operating temperature of 65℃, pressure of 60 bars(1bar = 10^5 Pa) and flowrate at 0.6 mL/min.

2.4 Products yields

Glucose yield of total pretreatment (pretreatment efficiency) as well as hydrolysis of the solid, cellulose rich fraction (to assess the availability of cellulose structure for enzymes actions) were calculated according to the following formulas:

$$\text{Hydrolysis yield} = \frac{\text{Glucose after hydrolysis[g]}}{\text{Glucose in raw material [g]}} \times 100\% \quad (1)$$

$$\text{Clean fractionation total efficiency} = \frac{(\text{Glucose in solid [g]}) + (\text{Glucose in aq. fraction [g]})}{\text{Glucose in raw material [g]}} \times 100\% \quad (2)$$

Glucose yield represents the ratio of the amount of glucose which can be recovered from the pretreated material to the amount of glucose in the material fed to the process.

Xylose represents about 80% of the hemicellulose fraction and is its only component currently considered as valuable. Therefore, due to very low amounts of arabinose, galactose and mannose found in the raw PCG, xylose was the only hemicellulose sugar analyzed. Xylose yield calculation is expressed by Equation (3). Lignin containing organic residual was not analyzed for purity at this point of research, since the main purpose of the study was optimization mainly for hydrolysis glucose yield. The organic fraction residual recovery was calculated based on the weight measurements, to estimate its potential in lignin extraction [Equation (4)]. However, to evaluate the extract's usability, its exact lignin content and quality would have to be analyzed.

$$\text{Xylose recovery} = \frac{\text{Xylose after hydrolysis/in aqueous fraction [g]}}{\text{Xylose in raw material [g]}} \times 100\% \quad (3)$$

$$\text{Lignin recovery} = \frac{\text{Lignin after CF [g]}}{\text{Lignin in raw material [g]}} \times 100\% \quad (4)$$

2.5 Response surface analysis

All the statistical analyses were performed using Design Expert version 8.0.1.0. Pretreatment experimental plan was based on a central composite experimental design (CCD). Small design with four replications of the center point was chosen, while all the points were performed in duplicates (Table 2). This resulted in 20 design points, with α-value of 1.681, chosen to ensure rotatability.

Table 2 Experiment plan for clean fractionation optimization

Experiment No.	Temperature /℃	Coded value	Time /min	Coded value	Ethyl acetate concentration %w/w]	Coded value	Ethanol concentration [%w/w]	Coded value
1	110.0	-1.00	10.0	-1.00	15.0	-1.00	10.0	-1.00
2	140.0	+1.00	10.0	-1.00	15.0	-1.00	10.0	-1.00
3	110.0	-1.00	30.0	+1.00	15.0	-1.00	35.0	+1.00
4	140.0	+1.00	30.0	+1.00	15.0	-1.00	35.0	+1.00
5	110.0	-1.00	30.0	+1.00	50.0	+1.00	10.0	-1.00
6	140.0	+1.00	30.0	+1.00	50.0	+1.00	10.0	-1.00
7	110.0	-1.00	10.0	-1.00	50.0	+1.00	35.0	+1.00
8	140.0	+1.00	10.0	-1.00	50.0	+1.00	35.0	+1.00
9	125.0	0.00	20.0	0.00	32.5	0.00	22.5	0.00
10	125.0	0.00	20.0	0.00	32.5	0.00	22.5	0.00
11	125.0	0.00	20.0	0.00	32.5	0.00	22.5	0.00
12	125.0	0.00	20.0	0.00	32.5	0.00	22.5	0.00
13	125.0	0.00	20.0	0.00	61.9	+1.68	22.5	0.00
14	125.0	0.00	20.0	0.00	3.1	-1.68	22.5	0.00
15	125.0	0.00	20.0	0.00	32.5	0.00	43.5	+1.68
16	125.0	0.00	20.0	0.00	32.5	0.00	1.5	-1.68
17	150.0	+1.68	20.0	0.00	32.5	0.00	22.5	0.00
18	100.0	-1.68	20.0	0.00	32.5	0.00	22.5	0.00
19	125.0	0.00	37.0	+1.68	32.5	0.00	22.5	0.00
20	125.0	0.00	3.0	-1.68	32.5	0.00	22.5	0.00

3 Results and discussion

3.1 Clean fractionation

The processing conditions had an influence on several response variables (glucose conversion, xylose recovery, lignin recovery, and by-products generation). The most important criteria in optimization were glucose conversion, lignin recovery, and xylose recovery. Another important aspect was using the mildest conditions, meaning lowest possible temperature and organic solvent concentration. Catalyst was eliminated to allow easier use of the xylose in the aqueous fraction, by avoiding neutralization. Figure 1 shows the comparison between conversion of cellulose into glucose among all 20 experiments, with distinction between hydrolysis glucose yields and overall pretreatment efficiencies in terms of glucose yield. As it can be seen from the Figure 1 the maximum hydrolysis conversion of cellulose into glucose (yield) was found in experiment 2 (42%) - with high temperature (140℃) but low chemicals concentration and short reaction time. The highest overall clean fractionation efficiency (58%) occurred in experiment 11. However, conversion of cellulose into glucose was more important since the aqueous fraction would not likely be used in glucose fermentation. The graph of monitored hydrolysis is shown in Figure 2 for two considered experiments (2 and 19).

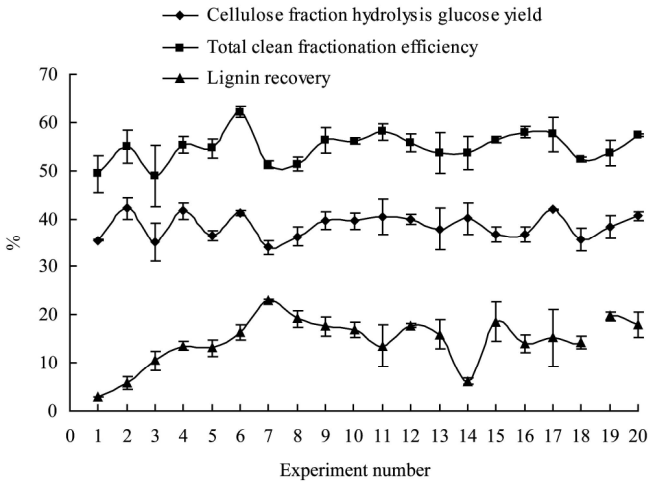

Figure 1 Glucose yield, process efficiency and lignin recovery changes throughout the experiments

Lignin containing residual yield was measured on a weight basis, after evaporation of ethanol and ethyl acetate from the extracted organic fraction. The results of this analysis can be found in Figure 1.

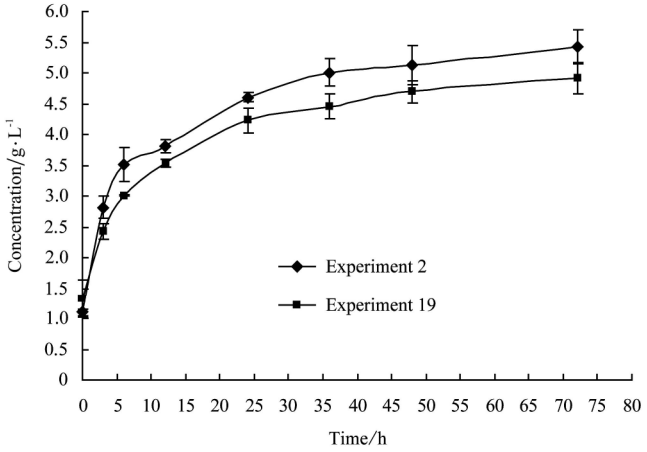

Figure 2 Glucose release during hydrolysis

The highest lignin recovery was obtained in experiment 7 (23%), at 140℃ for 10 min and using 50% w/w of ethyl acetate content. Lignin yields from clean fractionation span between 18%-32% (depending on the material type)[14]. Using green liquor extraction of hardwood chips, Luo et al.[24] obtained about 2.36% to 2.94% of lignin from raw wood materials. The lignin recovery in this study was resulted from low harshness process conditions at low temperatures without using inorganic acids. The processes used in this study can eliminate costly downstream waste water treatment and inorganic acid recycling.

It can be seen that experiment 7 resulted in about 8% lower glucose yield during hydrolysis (34% glucose yield and 51% glucose conversion for the entire pretreatment) than experiment 2 which produced the highest hydrolysis glucose yield. The second best result for lignin recovery was experiment 19 (20%), with 38% glucose yield during hydrolysis and 54% glucose conversion for the entire pretreatment. Furthermore, in this experiment about 20% w/w of the ethyl acetate was used (compared to experiment 7), with lower temperature (125℃), however with longer reaction time (37 min).

A third criterion of evaluation was xylose recovery, especially its extraction into the aqueous fraction. The aqueous fraction extracted at low temperatures was relatively clean, and this gave an opportunity of easy cleaning process (Table 3). The only contamination to be removed were the remaining organic solvents (ethyl

acetate and ethanol), which have similar boiling points (ethyl acetate – 77℃ and ethanol – 78℃). There was no furfural or HMF (hydroxy-methyl furfural) present in either the hydrolysis mixture or in the aqueous fraction. Low concentrations of acetic acid were formed, with the lowest values produced under the conditions of experiment 19.

Table 3 Concentration of acetic acid in the hydrolyzates and in aqueous fractions

Experiment No.	Acetic acid in hydrolysis of cellulose rich fraction /g·L^{-1}	Std. dev.	Acetic acid in aqueous fraction /g·L^{-1}	Std. dev.
1	0.33	0.09	0.00	0.00
2	0.29	0.02	0.55	0.05
3	0.26	0.01	0.00	0.00
4	0.29	0.01	0.00	0.00
5	0.31	0.04	0.00	0.00
6	0.36	0.01	0.00	0.00
7	0.31	0.07	0.00	0.00
8	0.29	0.02	0.00	0.00
9	0.31	0.03	0.00	0.00
10	0.29	0.01	0.00	0.00
11	0.30	0.01	0.00	0.00
12	0.29	0.00	0.00	0.00
13	0.34	0.09	0.00	0.00
14	0.32	0.04	0.00	0.00
15	0.29	0.01	0.00	0.00
16	0.27	0.01	0.47	0.05
17	0.31	0.03	0.27	0.39
18	0.28	0.01	0.00	0.00
19	0.19	0.06	0.00	0.00
20	0.26	0.04	0.00	0.00

Xylose recovery in the hydrolysis (representing xylose retained in the solid fraction) and xylose recovery in aqueous fraction can be found in Figure 3.

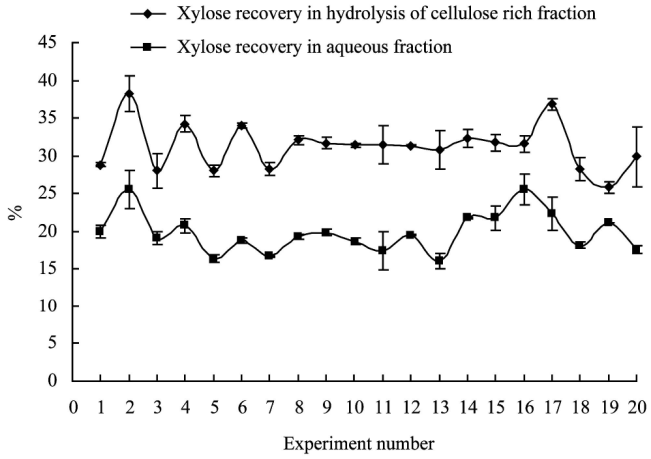

Figure 3 Xylose recoveries in the hydrolyzates and aqueous fractions

The highest overall xylose recovery (38% for hydrolysis and 26% for aqueous fraction) was produced by experiment 2 (15:10:75 solvent formulation, 140℃ and 10 min). Experiment 2 also produced the highest glucose yield. However, these same conditions produced very low lignin extraction (6%) and the process was carried out at the highest temperature (140℃). In case of formerly chosen conditions (experiment 19) xylose recovery was 26% for the hydrolysis of cellulose rich fraction and 21% for aqueous fraction. Hydrolysis was monitored for xylose production for experiments 2 and 19, and the results can be seen in Figure 4.

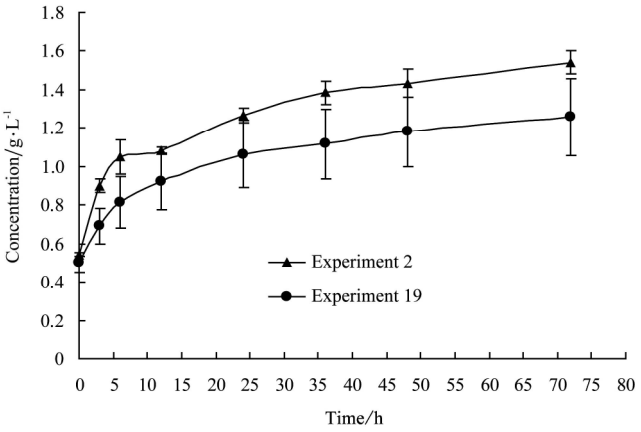

Figure 4 Xylose release during hydrolysis

Taking into account all the response variables discussed above, experiment 19th conditions were chosen as optimal (being one of the solutions of optimization found by the statistical software).

3.2 Response surface analysis

First and second order polynomial equations were developed to describe the relationship between four independent variables and six response variables. The regression equations can be found below with independent variable X_1 as ethyl acetate (w/w %), X_2 as ethanol (w/w %), X_3 as temperature (℃) and X_4 as time (min). Response variables were denoted as follows: Y_1 as glucose hydrolysis yield, Y_2 as glucose pretreatment efficiency, Y_3 as xylose hydrolysis recovery, Y_4 as xylose aqueous fraction recovery, Y_5 as acetic acid concentration after hydrolysis and Y_6 as lignin recovery.

$$Y_1 = 39.72 - 0.75X_1 + 0.02X_2 + 2.23X_3 - 0.69X_4 - 1.49X_1X_2 - 0.79X_1X_3 + 1.05X_1X_4 - 0.33X_2X_3 - 0.32X_1^2 - 1.12X_2^2 - 0.38X_3^2 \quad (5)$$

$$Y_2 = 56.18 + 0.03X_1 - 0.78X_2 + 3.26X_3 - 1.10X_4 - 2.06X_1X_2 - 2.08X_2X_4 + 0.23X_3X_4 - 0.88X_1^2 + 0.36X_2^2 - 1.45X_3^2 \quad (6)$$

$$Y_3 = 31.47 - 0.46X_1 + 0.02X_2 + 2.53X_3 - 1.66X_1X_2 - 0.44X_2X_4 + 0.56X_3X_4 + 0.02X_1^2 + 0.06X_2^2 + 0.36X_3^2 - 1.29X_4^2 \quad (7)$$

$$Y_4 = 19.67 - 1.91X_1 + 0.92X_2 + 1.11X_3 + 0.78X_4 \quad (8)$$

$$Y_5 = 0.29 + 0.01X_1 + 0.01X_2 + 0.01X_3 - 0.02X_4 + 0.01X_1X_3 + 0.02X_2X_4 + 0.02X_3X_4 + 0.01X_1^2 - 0.02X_4^2 \quad (9)$$

$$Y_6 = 0.17 + 0.03X_1 + 0.02X_2 - 0.001X_3 - 0.01X_4 + 0.01X_1X_2 - 0.01X_2X_4 + 0.02X_3X_4 - 0.02X_1^2 - 0.01X_3^2 \quad (10)$$

Values of R^2 (Table 4) showed that the models for each response variable were well fitted to explain the relationships among the variables. Also, the corresponding ANOVA tables for glucose yield in the hydrolysis, xylose aqueous fraction recovery and lignin recovery can be seen in Tables 5, 6 and 7.

Table 4 R^2 values for analyzed response variables

	Glucose Hydrolysis/ Pre-treatment	Xylose Hydrolysis/Aq. fraction	Acetic acid Hydrolysis	Lignin
R^2	0.98/0.97	0.99/0.90	0.96	0.92

Table 5 ANOVA for hydrolysis glucose yield

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	117.428	11	10.68	28.58	< 0.0001
A-Ester	7.626	1	7.63	20.42	0.0020
B-Ethanol	0.002	1	0.00	0.01	0.9400
C-Temperature	67.881	1	67.88	181.74	< 0.0001
D-Time	2.690	1	2.69	7.20	0.0278
AB	7.389	1	7.39	19.78	0.0021
AC	5.000	1	5.00	13.39	0.0064
AD	3.643	1	3.64	9.75	0.0142
BC	0.873	1	0.87	2.34	0.1648
A^2	1.477	1	1.48	3.95	0.0819
B^2	18.167	1	18.17	48.64	0.0001
C^2	2.096	1	2.10	5.61	0.0453
Residual	2.988	8	0.37		
Lack of Fit	2.431	5	0.49	2.62	0.2289
Pure Error	0.557	3	0.19		
Total	120.416	19			

Table 6 ANOVA for xylose in aqueous fraction

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	50.62	4	12.66	22.05	< 0.0001
A-Ester	35.21	1	35.21	61.35	< 0.0001
B-Ethanol	5.73	1	5.73	9.98	0.0102
C-Temperature	12.50	1	12.50	21.77	0.0009
D-Time	5.55	1	5.55	9.67	0.0111
Residual	5.74	10	0.57		
Lack of Fit	4.97	8	0.62	1.61	0.4380
Pure Error	0.77	2	0.39		
Total	56.36	14			

Table 7 ANOVA for lignin

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	0.031347	9	0.003483	11.45	0.0006
A-Ester	0.004759	1	0.004759	15.65	0.0033
B-Ethanol	0.004885	1	0.004885	16.06	0.0031
C-Temperature	0.000003	1	0.000003	0.01	0.9186
D-Time	0.000144	1	0.000144	0.47	0.5090
AB	0.000387	1	0.000387	1.27	0.2882
BD	0.000309	1	0.000309	1.02	0.3398
CD	0.002203	1	0.002203	7.24	0.0248
A^2	0.006415	1	0.006415	21.09	0.0013
C^2	0.000971	1	0.000971	3.19	0.1076
Residual	0.002737	9	0.000304		
Lack of Fit	0.001551	6	0.000258	0.65	0.6997
Pure Error	0.001187	3	0.000396		
Total	0.034084	18			

From the ANOVA tables (Tables 5, 6 and 7), it can be seen that temperature and ester (ethyl acetate) content had the strongest influence on the glucose yield (p-values <0.05). Temperature increase induces cleavage of the lignin-carbohydrates bonds, while ethyl acetate content promotes delignification, proven to influence cellulose digestibility[22]. The same observation was noted for xylose extraction to aqueous fraction – recovery was strongly dependent on temperature and ester content. For lignin recovery, the strongest effect was shown by ester and ethanol content in the solvent, as well as the interaction between time and temperature. Predicted optimal conditions for the clean fractionation were developed based on the regression equations.

The response surface graphs in Figure 5 showed that in fact temperature and ester content had major effects on both glucose yield and xylose extraction to aqueous

fraction. The organic components ratio in the solvent had a major effect on lignin recovery. Glucose yield increased with increasing temperature with favorable low level of ester. Xylose extraction showed the same trend. Glucose yield was influenced more by temperature than ester content. The trend was opposite in xylose recovery. Response surface plots for lignin recovery showed that the interaction effect between ethanol and ester had an effect on the lignin recovery, but only at high ester contents. The highest lignin recovery occurred at high ethanol and ester contents. Time and temperature interaction was also significant (confirmed by p-value < 0.05).

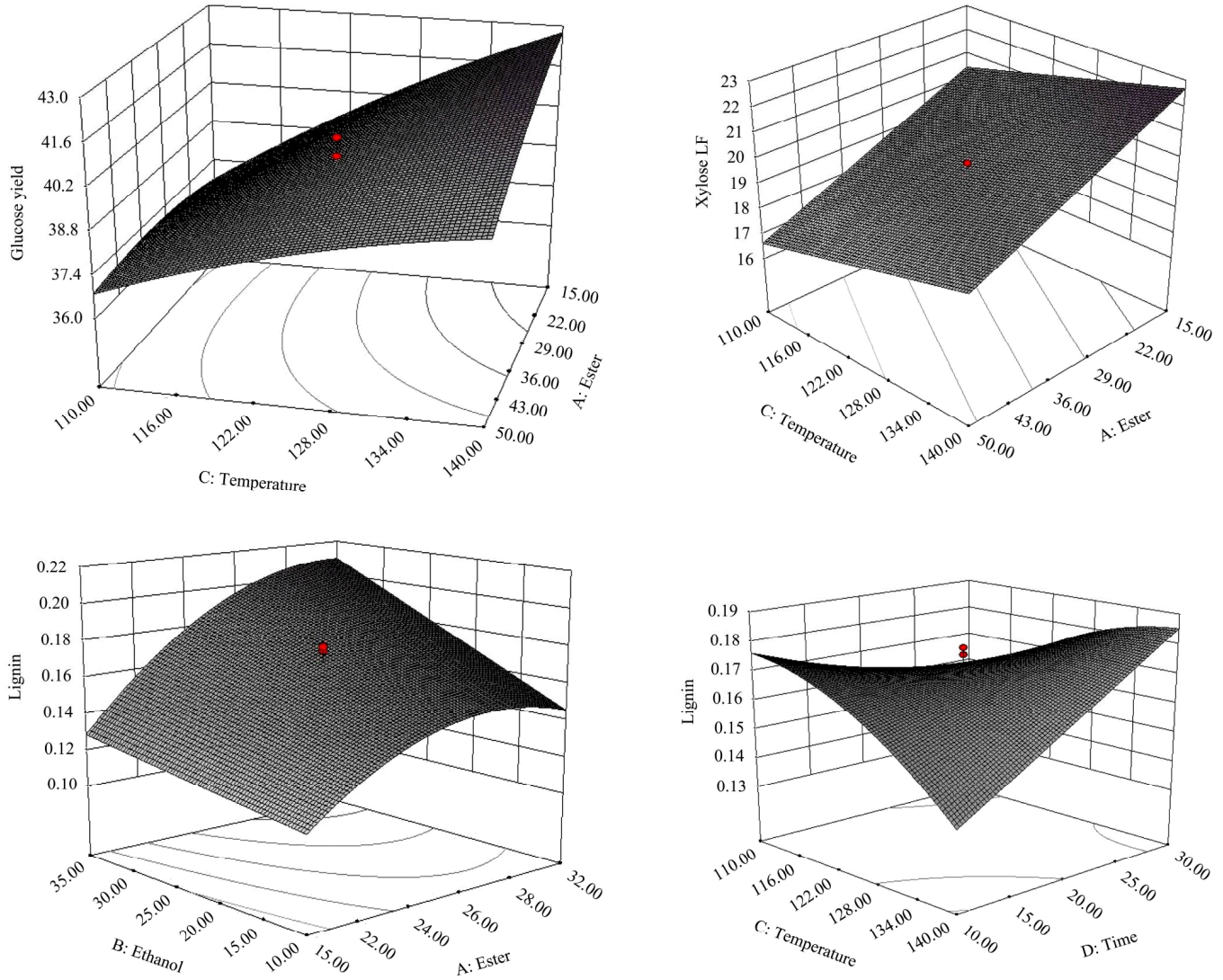

Figure 1 Response surface graphs for glucose yield, xylose recovery and lignin recovery in clean fractionation

4 Conclusions

The results showed that clean fractionation using ethyl acetate as the organic solvent can be an effective process for removal of lignin from lignocellulosic biomass. However, the recovery of organic solvent soluble lignin was as low as 20%. This low result could be enhanced with addition of catalyst. Usage of catalyst (e.g. sulfuric acid) in the NREL optimized process significantly enhanced cellulose hydrolytic digestibility, when added in high concentration (over 5% per biomass DM)[14]. The same results for glucose yields were achieved by applying a short hydrothermal post fractionation treatment (up to 90% yield in the hydrolysis)[22]. Thus the mineral catalyst can be eliminated from the process, but only if glucose is a main desired product.

Several criteria were considered to evaluate the optimal conditions for the clean fractionation step: lignin recovery, xylose recovery in the aqueous fraction and glucose yield during the hydrolysis. Also, the mildest

possible conditions were chosen to eliminate high temperatures and chemicals usage. The optimal conditions determined for the clean fractionation were 125℃, 37 min, with the solvent composition equal to ester:ethanol:water = 32.5:22.5:45. This resulted in a 20% recovery of organic solvent soluble lignin, 21% of xylose recovery in aqueous fraction and 38% glucose yield during the hydrolysis. The aqueous fraction was free of the typical pretreatment by-products; however the remaining solvent has to be removed in order to utilize this fraction as xylose fermentation substrate.

Analysis of variance showed that in all experiments temperature had a significant effect on the output, producing the best results at values near the center point. Clean fractionation experiments revealed ethyl acetate content in the solvent significantly influenced all the response variables, while the best results were achieved at the center point values.

Further work would have to be done to evaluate the applicability of extracted lignin fraction, which includes analysis of Klason lignin, ash content, and possibly analysis of functional groups and molecular weight. At this point, the extracted fraction containing lignin represents too low quantity in order to be a potential valuable product. Moreover, in order to evaluate the economical feasibility of the process, an up-scaling with solvents recovery would have to be performed.

Acknowledgements

This work was supported by funding from the South Dakota Center for Bioprocessing Research and Development, Sun Grant Initiative and SDSU Agricultural Experiment Station.

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