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Delignification of Intact Biomass and Cellulosic Coproduct of Acid-Catalyzed Hydrolysis

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The kinetics of acid-catalyzed hemicellulose removal and also alkaline delignification of oat hull biomass were investigated. All three operational parameters namely, catalyst concentration (0.10–0.55 N H_2SO_4), temperature (110–130°C), and residence time (up to 150 min) affected the efficiency of hemicellulose removal, with 100% of hemicellulose removed by appropriate selection of process parameters. Analysis of delignification kinetics (in the temperature range of 30–100°C) indicated that it can be expressed very well by a two-phase model for the crude biomass and also for the hemicellulose-prehydrolyzed material. The application of acid-catalyzed prehydrolysis improved the capacity of lignin dissolution especially at lower temperatures (30 and 65°C) and accelerated the dissolution of lignin. This acceleration of delignification by prehydrolysis was possible at all levels of temperature in the bulk phase; however, results were more significant at the lower temperatures in the terminal phase. © 2015 American Institute of Chemical Engineers *AIChE J*, 61: 1783–1791, 2015

Keywords: lignocellulose, lignin, delignification, hydrolysis, biomass

Introduction

Hemicellulose and lignin are the two polymers that exist with cellulose in the structure of lignocellulosic materials. Hemicelluose is a plentiful polysaccharide in nature which is ranked the second after cellulose in terms of abundance.[1] Because of its heterogeneous composition, it is sometimes termed hemicelluloses, which is a group of polysaccharides.[2] There has been increasing interest in xylan, the main polymer in hemicellulose with the backbone of β-(1–4)-linked-D-xylopyranosyl units (shown in Figure 1), because of its wide ranging potential application in the pharmaceutical, food, and cosmetic industries.[3–7]

For the production of cellulose-based products, the removal of lignin from the biomass by the application of biological, chemical, or physical pretreatments could improve the exposure of cellulose to biocatalysts and the recovery of carbohydrates for biochemical applications.[8] Among all pretreatments, the chemical methods (acid pretreatment, alkaline pretreatment, ammonia fiber/freeze explosion, organosolv, and ionic liquids pretreatment), especially acid pretreatment is the most favored method for the consequent biocatalytic conversion of cellulose.[9,10] Alkaline pretreatment is considered an effective delignification method by which a great part of hemicellulose is also removed from the lignocellulosic matrix through solubilization based on saponification of ester bonds in the hemicellulose or between hemicellulose and lignin. Biomass swelling, lignin modification, decreased cellulose crystallinity, and degree of polymerization, and increased internal surface area result from alkaline pretreatment of biomass.[10] Many research studies have been conducted to enhance delignification of lignocellulosic biomass. A xylanase-catalyzed reaction was used for lignin removal from softwood in a batch system. Experimental results and modeling of the process indicated an increased level of delignification with up to 48% lignin removal using a lower molecular weight xylanase.[11] In a kinetic study using a continuous stirred tank reactor, an accelerated delignification was achieved by the increased levels of oxygen pressure in alkaline delignification process. Analytical results indicated that adsorption of oxygen on the active aromatic lignin site follows a Langmuir-type behavior.[12] An effective lignin removal at ambient temperature with less delignifying agent was attained in a simulation study using ultrasound-assisted delignification of biomass under alkaline condition.[13] In oxygen delignification of pine kraft pulp, the use of a continuous reactor resulted in a significantly higher performance compared with a batch reactor.[14] In a research study evaluating lignocellulose biotreatment, a faster and shorter initial delignification phase was reported in the fungi-treated wood.[15] An organosolv pulping using formic acid resulted in a highly effective lignin removal with a minor dissolution of other components from bamboo.[16] Heterogeneous modeling of wood pulping indicated that the resistance of boundary layer surrounding the wood is an important limiting factor in lignin dissolution which needs to be considered to improve lignin removal.[17]

Chemical pretreatments could also be used to improve the physical and chemical properties of cellulose-based fibers.

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Application of cellulose-based fibers in biocomposites could have several mechanical and thermal advantages. In biofiber-based composites, the presence of higher cellulose fraction in the fiber can lead to a higher modulus of elasticity of the product[18] and can increase the onset temperature of degradation as well as degradation temperature.[19] In cellulosic fiber-gluten composites, higher glass transition temperature and higher modulus of elasticity but lower water absorption were observed compared with gluten-based material; less lignin in the fiber resulted to decreased levels of water absorption.[20]

Oat hull is a biomass which is rich in hemicellulose (37%) and cellulose (35%).[21] The global annual production of oat hull is over 20 million tons, and it is produced in large quantity (over 3 million tons) in Canada.[22,23] Almost a quarter of the incoming raw material entering an oat processing plant ends up as oat hull. Oat hulls are presently marketed as low-quality and low-value animal feed. With its high cellulose and hemicellulose content, it has potential applications in bio-based products such as biofuel, biochemicals, and bicomposites. Hemicellulose as a source of xylan can be used for production of high value products such as xylose, xylitol and furfural; cellulose can be used for the production of cellulose-based composites or biofuels and bioproducts.

Although a great number of efforts have been made to improve delignification performance as mentioned above, there is still a knowledge gap related to the effect of the full removal of hemicellulose on the improvement and the kinetics of delignification. A positive effect of hemicellulose removal on the subsequent step of delignification can be used as a dual purpose strategy in a biorefinery, that is, extraction of hemicellulose for xylan-/xylose-based products, and the accelerated delignification with less energy consumption and residence time for a cellulosic coproduct. Furthermore, knowledge of the kinetics of hemicellulose removal and delignification of cellulosic biomass, specifically oat hulls in this work, would be helpful in the selection of process parameters to provide a cellulosic material with a specific composition for a specific application. The objectives of this work are: (1) to investigate the behavior of (acidic) hemicellulose removal under process parameters and to optimize the parameters for full removal of hemicellulose; and (2) to investigate the influence of biomass prehydrolysis (for full removal of hemicellulose) on delignification efficiency and to compare the results with delignification of untreated biomass in achieving higher cellulose content in the coproduct.

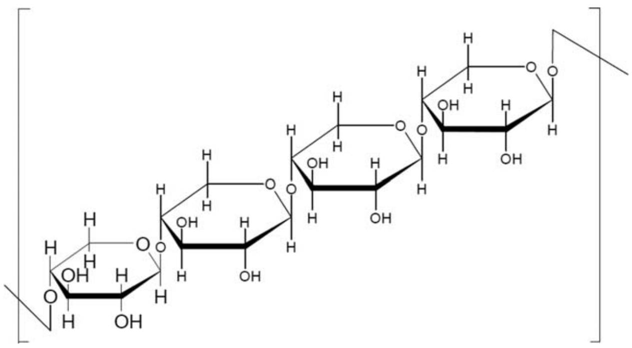

Figure 1. Chemical structure of xylan (polymer of β-(1–4)-D-xylopyranosyl).

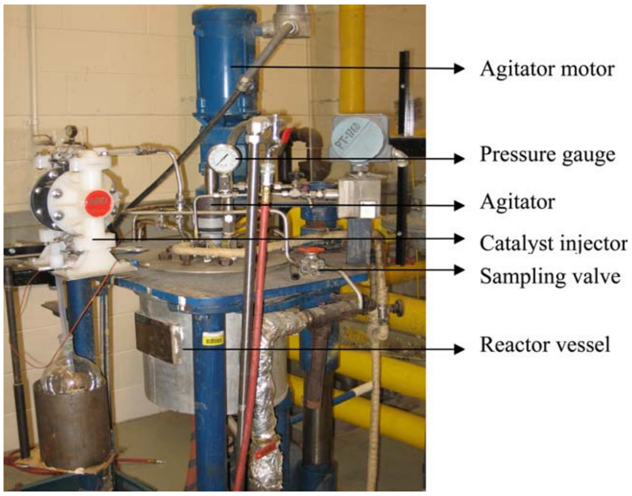

Figure 2. Process reactor used for the hydrolysis of oat hull.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Materials and Methods

Oat hull as the raw material of these experimental trials was supplied by Can-Oat Milling (now Richardson Milling Ltd., Martensville, SK); impurities including endosperm grits and dust were separated using a sieving machine (Link Aero, Fargo, ND). For acid detergent fiber (ADF) determinations, sulfuric acid and cetyl trimethyl ammonium bromide (CTAB) were supplied by Sigma Aldrich Canada (Oakville, ON), and acetone was supplied by VWR (Vancouver, BC). For neutral detergent fiber (NDF) measurements, α-amylase, ethylene diamine tetra acetic acid, sodium lauryl sulfate, sodium sulfite, and triethylene glycol were supplied by Sigma Aldrich (Oakville, ON). Sodium hydroxide was obtained from VWR (Vancouver, BC) for delignification experimental work.

The hydrolysis reactions for the removal of hemicellulose from oat hull samples were performed using a 4-L process reactor ([Hoppes, Springfield, OH] Figure 2) to provide pressurized condition (temperature of 130°C). This was a jacketed reactor in which the pressure/temperature was controlled by the steam pressure in the jacket. It was also equipped with an agitator for homogenizing the suspension and to provide a uniform solid: liquid ratio inside the vessel. Hemicellulose removal was conducted with variables of temperature (110, 120, and 130°C), sulfuric acid concentration (0.10, 0.25, 0.40, and 0.55 N), and residence time up to 150 min with sampling every 5 min at the beginning and every 10 min after 20 min progress of the process. Each set of operation was performed in three replicates. The solid fraction of the samples was filtered out for the consequent measurement of hemicellulose content after drying. The alkaline method of delignification using 5% sodium hydroxide at temperatures of 30, 65, and 100°C under reflux was used with three replicates. For delignification process, a 2-L beaker on top of a magnetic stirrer hot plate (IKA, Wilmington, NC) with temperature control was used as the reaction vessel. The beaker was covered with a moulded silicon-based resin with three holes; one in the middle for a condenser for reflux and to keep the concentration of the alkaline solution and also the ratio of alkaline solution: biomass constant during delignification; the other two holes were considered for thermocouple sensor and the sampler tube. Delignification experiments were conducted in triplicate on the oat hull and the prehydrolyzed (at 0.1 N catalyst (H_2SO_4) concentration) oat hull samples, separately for total residence time of 150 min and sampling intervals of 5 min within the 20 min beginning of the process and 10-min sampling intervals afterward. Sampling was performed carefully by putting a glass pipe into the suspension to suction both liquid and solid fractions during agitation to keep the solid-to-liquid ratio constant during the process.

The hemicellulose and cellulose contents were determined based on NDF and ADF following AOAC standards No. 2002.04[24] and No. 973.18,[25] respectively. Klason lignin and acid soluble lignin (ASL) were measured, respectively, by NREL standard.[26]

Briefly, for the NDF method, two solutions namely, enzyme and neutral detergent solutions were used, respectively, for digestion of starch and digestion and dissolution of wax, proteins, lipids, and nonfibrous carbohydrates. After digestion of the samples, the nonsoluble part was filtered and washed thoroughly with boiling distilled water and then was dried in a convection oven (Despatch Oven Co., Minneapolis, MN) at 105°C for 8 h. The dried sample was incinerated at 500°C for 1 h and finally the difference of weights before and after incineration gave the NDF amount. ADF was determined by refluxing samples in 1.0 N sulfuric acid solution containing CTAB for 1 h. Finally, it was filtered and washed thoroughly with distilled water and acetone and dried in the oven at 100°C for 3 h.

For lignin quantification, a gravimetric method was also used. Samples were predigested in a 72% sulfuric acid solution (15°C) for 2 h and digested with boiling diluted acid (3%) under reflux for 4 h. At the end, the extract was used to determine ASL and the solid fraction after filtration and drying at 105°C was used to determine Klason lignin.

Kinetics of delignification

Delignification of biomass could be performed in three stages: initial, bulk, and residual. In the initial stage of alkaline pulping, α-aryl ether linkages are cleaved at a high rate. In the bulk stage, which is slower than initial phase, most of the lignin is removed and its reaction rate depends on the cleavage of β-aryl ether linkages. In the terminal stage or residual period, the cleavage of carbon–carbon bonds in lignin molecule as well as carbohydrate degradation occurs.[27,28]

Lignin removal or dissolution rate could be expressed as a first-order mechanism

$$\frac{dL}{dt}=-kL \tag{1}$$

where L is lignin content (g/L) at time t (min) and k (L/min) is the kinetic constant of the reaction. Assuming the presence of only one type of lignin, the activation energy would be in the range of 66.5–78.2 kJ/mol.[29] However, according to the more common assumption, different types of lignin react sequentially as initial, bulk, and residual stages. Based on this mechanism, which is called nonreversible three-stage delignification, the reaction could be expressed as

$$\mathrm{L_{N1}}\xrightarrow{k_1}\mathrm{L_{D1}} \tag{2}$$

$$\mathrm{L_{N2}}\xrightarrow{k_2}\mathrm{L_{D2}} \tag{3}$$

$$\mathrm{L_{N3}}\xrightarrow{k_3}\mathrm{L_{D3}} \tag{4}$$

L_{N1}, L_{N2}, and L_{N3} are the three fragments of the native lignin reacting in different rates and L_{D1}, L_{D2}, and L_{D3} are the dissolved forms of lignin fragments

$$L=a\cdot\exp(-k_1\cdot t)+b\cdot\exp(-k_2t)+c\cdot\exp(-k_3\cdot t) \tag{5}$$

where a, b, and c are the maximum values of lignin released in initial, bulk, and residual stages:

$$a+b+c=1 \tag{6}$$

In addition to the three-phase mechanism, the two-stage (Eq. 7; excluding initial stage) and single-phase (Eq. 8; excluding initial and bulk stages) mechanisms were also considered to analyze the results

$$L=b\cdot\exp(-k_2t)+c\cdot\exp(-k_3\cdot t) \tag{7}$$

$$L=c\cdot\exp(-k_3\cdot t) \tag{8}$$

The activation energy (E_a) of the components was calculated using the Arrhenius equation

$$k=k_0\cdot\exp\left(\frac{-E_a}{RT}\right) \tag{9}$$

where k_0 and E_a are, respectively, the preexponential factor and activation energy (kJ/mol), R is the universal gas constant (8.314×10^{-3} kJ/mol K), and T is the absolute temperature (K). Data obtained from chemical analysis of the samples were collected and analyzed using nonlinear regression (Proc NLIN) with the Newton's method by SAS (Statistical Analysis System, Cary, NC) and by minimizing the sum of the squares of deviations between experimental and calculated data from the models.

Experimental design and analysis

As delignification process is the main objective of this work, a full factorial statistical design with three replicates for all combinations (102) of the factors including temperature (at three levels of 30, 60, and 100°C), residence time (at 17 levels from 5 to 150 min), and the type of the material (at two levels including raw and prehydrolyzed biomass) was used. The quantitative results obtained for lignin and cellulose during delignification process as dependent variables were analyzed by the analysis of variance (ANOVA) using SAS (statistical analysis software) to determine how the factors and interactions of the factors affected these values. Then, the following first-order polynomial was fitted for each type of the materials (crude and prehydrolyzed biomass) separately to obtain the relationship between the dependent variables (lignin and cellulose) and the independent variables

$$y_i=\alpha_1(T)+\alpha_2(t)+\alpha_3(T\times t)+\beta \tag{10}$$

where y_i represents the response (dependent variables), T and t are, respectively, the process temperature and residence time, α_1, α_2, and α_3, are the regression coefficients, and β is the intercept. The coefficient of determination (R^2) was obtained to determine whether the regression models fitted the data well.

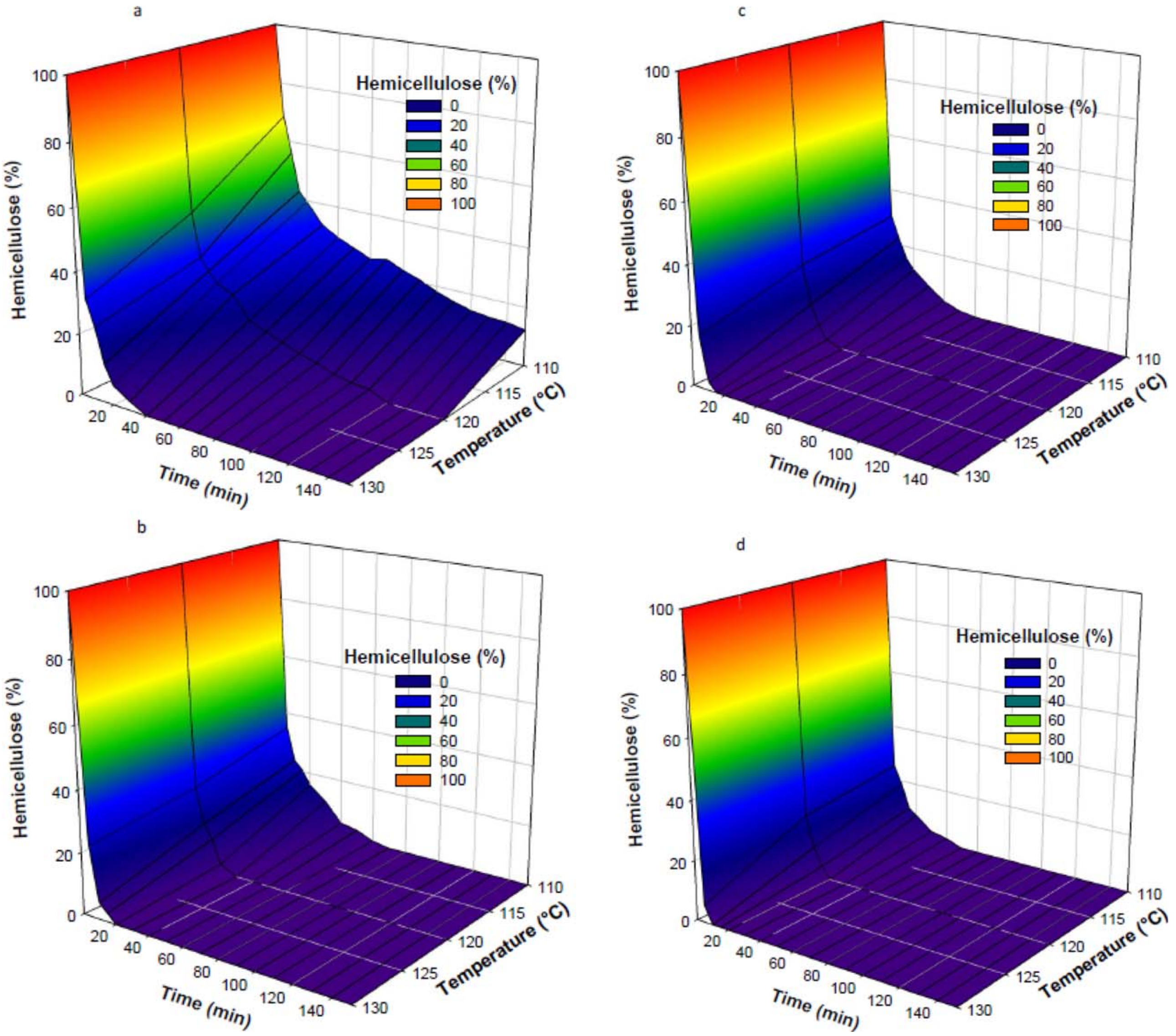

Figure 3. Hemicellulose removal from oat hull biomass as affected by time and temperature at catalyst concentrations: (a) 0.10 N, (b) 0.25 N, (c) 0.40 N, and (d) 55 N.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Results and Discussion

The analysis of oat hull biomass indicated that it was composed of 39.6% (w/w) hemicellulose, 30.9% (w/w) cellulose, 18.5% (w/w) Klason lignin, 3.2% (w/w) ASL, and 3.2% (w/w) starch. Therefore, it is a rich source of hemicellulose and cellulose. Sugar analysis indicated that 100 g biomass consisted of 40.5 g glucose, 37.5 g xylose, 4.2 g arabinose, 1.5 g galactose, and 0.7 g mannose. Glucose is the monomer of cellulose, and C5 sugars (xylose and arabinose) and the rest of the C6 sugars (galactose, mannose, and part of glucose) are the monomers of hemicellulose. As a result, the major sugar in the acid-catalyzed hydrolysate after hemicellulose extraction from the biomass would be xylose which is derived from polymer xylan. The hemicellulose removal causes the contents of cellulose and lignin to increase in the cellulosic coproduct which will be explained.

Hemicellulose removal

Acid-base titrations indicated that the catalyst (sulfuric acid) concentration did not change with time in all operating conditions. This could be due to the high ratio of the liquid phase to the solid phase and the high strength of the catalyst used in hydrolysis process for hemicellulose removal. Therefore, the release of low amounts of organic substances (such as acetyl group, which generates acetic acid with a low dissociation constant) during hemicellulose hydrolysis process did not significantly affect the concentration of hydrogen ion in the liquid phase. Extraction or removal of hemicellulose from oat hull samples using acid pretreatment is shown in Figure 3 at catalyst (H_2SO_4) concentrations of 0.10, 0.25, 0.40, and 0.55 N, respectively. Overall, it was observed that the removal of this biopolymer occurred in two stages which could be due to the presence of fast and slow releasing fractions of this polysaccharide. As shown in Figure 3, the

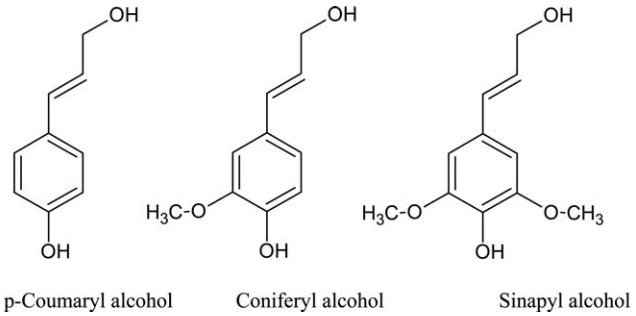

Figure 4. Phenylpropane-based monomers of lignin.[30]

hemicellulose content never reached zero (it reached an amount of about 12% in the treated biomass) under the mildest condition of the process (0.1 N catalyst and temperature of 110°C). However, at the same catalyst concentration, its extraction was accelerated by increasing temperature so that after 120 and 40 min, it was completely depleted at temperatures of 120 and 130°C, respectively. Comparison of the hemicellulose removal trend in Figure 3 indicates that catalyst concentration also functions as an accelerating agent for this purpose. For example, at a temperature of 130°C, the time required for complete extraction of this material dropped from 40 min to just 10 min at acid concentrations of 0.1 and 0.55 N, respectively. Therefore, the low extraction potential of low catalyst loading could be compensated by the use of higher temperature which makes the consequent stages such as neutralization much easier and less costly. For the next process of delignification, prehydrolyzed material obtained by the parameter combination catalyst concentration/temperature/residence time of 0.1 N/130°C/40 min was selected to perform lignin dissolution experimental work and kinetic analysis. This optimal combination of catalyst concentration/temperature/residence time (0.1 N/130°C/40 min) resulted in a cellulosic coproduct with enriched amounts of cellulose and lignin (52.4% cellulose and 32.1% lignin) after complete removal of hemicellulose.

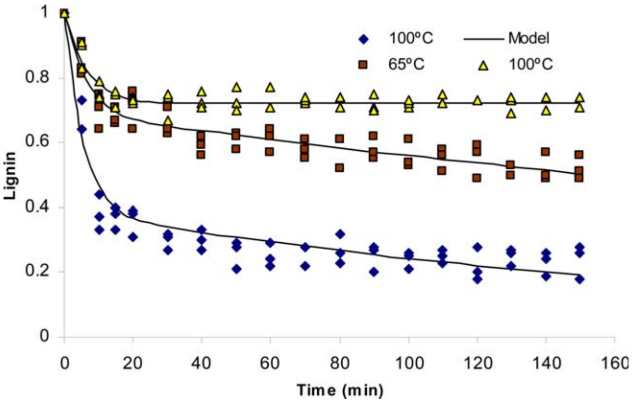

Figure 5. Delignification behavior of oat hull (crude biomass) at different temperatures graphed by data from experimental work and two-stage model.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

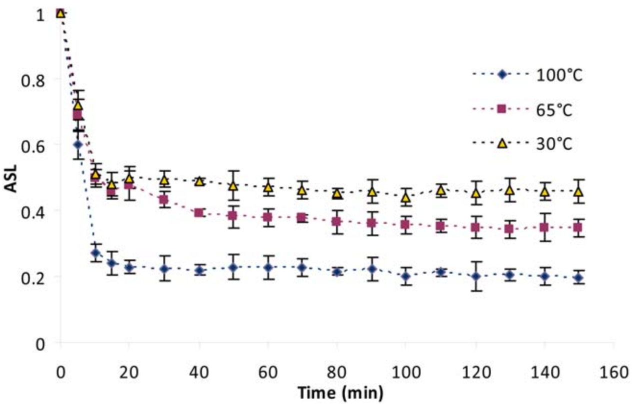

Figure 6. Removal of ASL from oat hull (crude biomass) at different temperatures.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Delignification of biomass

Lignin is a highly cross-linked macromolecule composed of phenylpropane building blocks that plays an important role in cell wall structure as a permanent bonding agent among plant cells. It is always associated with hemicellulose in the cell wall. The three forms of lignin monomers or precursors are shown in Figure 4.[30] The predominant component of lignin in softwood is coniferyl alcohol; while in hardwood, it is mainly based on coniferyl and sinapyl alcohols. In forage and grass, all three alcohols are incorporated to make building blocks of lignin.[31] The complexity of lignin structure is due to the various functional groups on phenylpropane units and the diversity of linkages between the units.

Some of the common linkages in lignin molecule are: phenylpropane β-aryl ether, phenylpeopane α-aryl ether, phenyl coumaran, biphenyl and dibenzodioxocin, diaryl ether. The dominant linkage is β-aryl ether (β-O-4) which accounts for 45–50% of interphenylpropane linkages. In terms of functional groups, lignin contains a variety of groups such as phenolic hydroxyl groups, methoxyl groups, and terminal aldehyde groups which are effective on its reactivity.

Acid-base titrations indicated that the catalyst (sodium hydroxide) concentration did not change with time in all

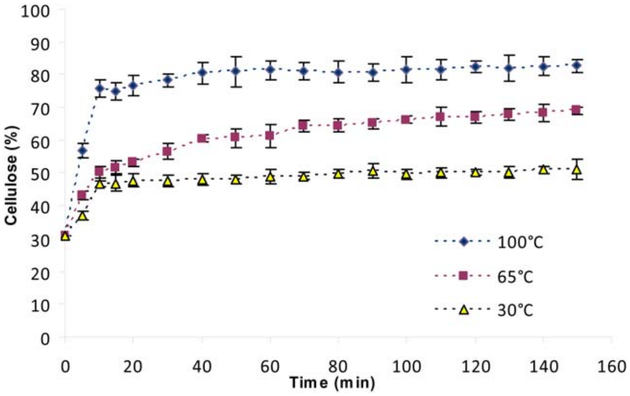

Figure 7. Cellulose content of oat hull (crude material) during delignification process at different temperatures.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 1. Parameter Estimates for Single-Stage as Well as Two- and Three-Stage Sequential Lignin Dissolution in Oat Hull (Crude Biomass)

Model	Temperature (°C)	a (g/g)	b (g/g)	c (g/g)	k_1 (L/min)	k_2 (L/min)	k_3 (L/min)	R^2
Single-stage	30	–	–	0.81	–	–	0.001120	0.99
	65	–	–	0.79	–	–	0.003530	0.98
	100	–	–	0.61	–	–	0.010900	0.88
Two-stage	30	–	0.28	0.72	–	0.151	0.000040	1.00
	65	–	0.31	0.69	–	0.163	0.002110	1.00
	100	–	0.61	0.39	–	0.189	0.004720	0.98
Three-stage	30	0.11	0.17	0.72	**0.155**	**0.155**	0.000002	1.00
	65	0.14	0.19	0.67	0.201	0.109	0.001730	1.00
	100	0.18	0.54	0.28	0.570	0.105	0.001250	0.99

k_1, k_2, and k_3, reaction rate constants; a, b, and c, lignin fragments; R^2, coefficient of determination. The (equal) bold numbers for the rate constants in three-stage model at lower temperature indicate that this is not an applicable model at 30°C.

Table 2. Parameter Estimates for Single-Stage as Well as Two- and Three-Stage Sequential Lignin Dissolution in Hemicellulose-Prehydrolyzed Biomass

Model	Temperature (°C)	a (g/g)	b (g/g)	c (g/g)	k_1 (L/min)	k_2 (L/min)	k_3 (L/min)	R^2
Single-stage	30		–	0.68		–	0.00174	0.98
	65		–	0.65		–	0.00562	0.95
	100		–	0.53		–	0.00913	0.87
Two-stage	30	–	0.44	0.56	–	0.253	0.00054	0.99
	65	–	0.46	0.54	–	0.270	0.00341	0.99
	100	–	0.63	0.37	–	0.545	0.00432	1.00
Three-stage	30	0.18	0.23	0.59	**0.319**	**0.319**	0.00239	1.00
	65	0.19	0.35	0.46	3.835	0.107	0.00177	1.00
	100	0.21	0.42	0.37	3.554	0.455	0.00427	1.00

k_1, k_2 and k_3, reaction rate constants; a, b, and c, lignin fragments. The (equal) bold numbers for the rate constants in three-stage model at lower temperature indicate that this is not an applicable model at 30°C.

operating conditions. This could be due to the high ratio of the liquid phase to the solid phase and the high strength of the catalyst used in delignification process. The release of phenol or phenol derivatives, which are weak organic acids, during delignification did not significantly change the concentration of hydroxyl ion originally generated from sodium hydroxide dissociation.

As shown in Figures 5 and 6, the removal rate and potential of both Klason and ASL are dependent on process temperature. For example, at temperatures of 30, 65, and 100°C, over 25, 40, and 70% of the initial Klason lignin (18.5% d.b.) was removed; this was over 50, 60, and 70% for ASL, respectively. The variation of cellulose in oat hull during delignification is shown in Figure 7 which could be improved to around 80% from 30.9% of the material at the highest temperature (100°C) tested.

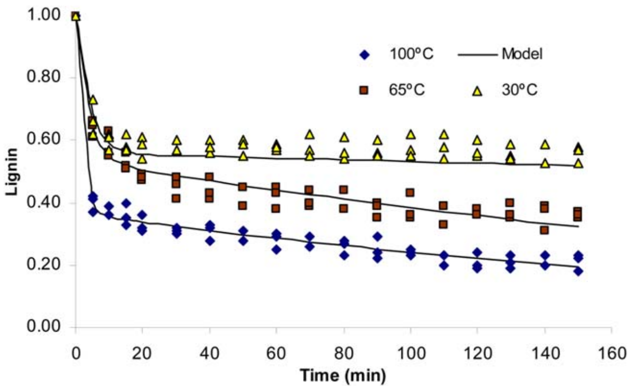

Figure 8. Delignification behavior of hemicellulose-prehydrolyzed biomass by data from experimental work and two-stage model.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The results of kinetic analysis based on the three mechanisms of lignin dissolution for intact (raw) biomass are tabulated in Table 1. Compared with the sequential mechanisms, single stage mechanism was less accurate in describing the results, especially at higher temperature as the coefficients of determination (R^2) show. In terms of accuracy in predicting the results, the two-stage and three-stage based equations are almost the same, however at 30°C, the same values (0.155) were obtained for k_2 and k_3 meaning that it could be considered as a two-stage reaction. Also, the variation of rate constants did not show a reasonable trend by temperature variations in the three-phase model as shown in Table 1. Therefore, the two-phase model is preferred over the other models. Kim and Holtzapple[27] also achieved better results using a two-phase model to characterize the kinetics of corn stover delignification using lime. As presented in Tables 1 and 2 and based on the two-stage model, by increasing temperature from 30 to 100°C, the values of b (maximum bulk phase lignin) increased and the values of c (maximum terminal phase lignin) decreased, respectively, from 0.28 to 0.61 and from 0.72 to 0.39 in oat hull (crude biomass); the corresponding variation values for the prehydrolyzed material are from 0.44 to 0.63 and from 0.56 to 0.37 for b and c, respectively. Therefore, increased process temperature resulted to the switch of delignification from the residual stage to the bulk stage for both kinds of materials. This agrees with the results reported by Kim and Holtzapple[27] and Sabatier

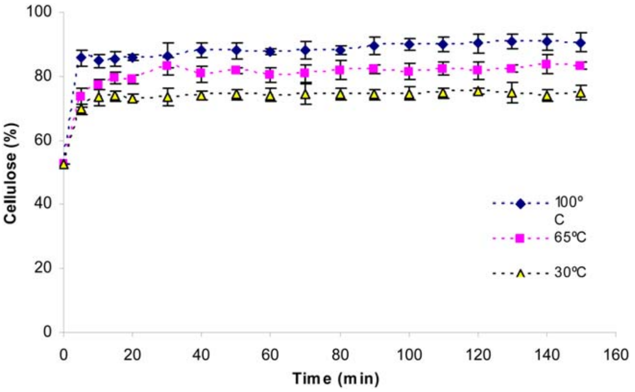

Figure 9. Cellulose content of the material (hydrolysis coproduct) during delignification process.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 3. Activation Energies of Delignification for Crude and Hemicellulose-Prehydrolyzed Biomass

Source	k_{02} (L/min)	k_{03} (L/min)	E_{a2} (kJ/mol)	E_{a3} (kJ/mol)
Crude biomass	0.44	80.47	2.71	31.21
Prehydrolyzed biomass	36.65	138.78	13.16	32.91

k_{02} and k_{03}, preexponential factors in the second and third stages, respectively; E_{a2} and E_{a3}, activation energies of delignification in the second and third stages, respectively.

et al.[32] who obtained increased delignification performance at the bulk stage than at the residual stage with the increased process temperature. Comparing the rate constants indicates that lignin dissolution rate in the bulk stage is more dependent on temperature for the crude biomass than the prehydrolyzed biomass as presented in Tables 1 and 2 due to the higher variations in k_2. However, the dissolution rate of lignin in the terminal stage is more dependent on temperature for the crude biomass compared with the prehydrolyzed biomass.

It is observed from the results in Tables 1 and 2 that higher values of rate constants for the prehydrolyzed material prove that hemicellulose hydrolysis would accelerate the delignification rate of biomass; this enhancement was significant at all levels of temperatures in the bulk phase, but was more significant at lower temperatures especially at 30°C in the terminal phase.

To investigate the influence of hydrolysis process on delignification performance of the biomass and to compare it with untreated material, some other experimental work were conducted and the results are graphed in Figures 8 and 9. Comparing the results under similar conditions for crude biomass and pretreated material (in Figures 5 and 8) indicates that acid pretreatment of the material improved the delignification performance, especially at lower temperatures (30 and 65°C) than at the highest temperature (100°C). For instance, at the lowest temperature of 30°C, lignin content was reduced by over 40% in the prehydrolyzed material; however, lignin removal was less than 30% in the crude material. As a consequence, better results were obtained for cellulosic fraction in prehydrolyzed material after delignification as indicated in Figure 9.

The activation energy of delignification based on a two-stage mechanism was determined for crude and hemicellulose-prehydrolyzed biomass; for both materials, the value of activation energy in the terminal (residual) step of dissolution was significantly higher than the ones obtained in the bulk stage (Table 3). The value of activation energy for both materials was almost the same at the terminal stage but in the first stage, it was less for the crude material. This could be due to the dependence of dissolution rate of lignin in the prehydrolyzed material on the process temperature as explained previously compared with the crude material. The values presented here, are smaller than the values reported for lignin removal in other studies. Kim and Holtzapple[27] reported activation energies of 70.24 and 10.74 kJ/mol under nonoxidative lime delignification of corn stover for the bulk and residual steps, respectively. These values were 50.15 and 54.21 kJ/mol for bulk and residual stages, respectively, under oxidative delignification. Activation energy for bagasse was 42 kJ/mol in a single-step reaction.[32] In wood pulping, higher numbers were reported for delignification; they were 120–130 and 110–117 kJ/mol, respectively, for bulk and residual stages.[33]

Therefore, the use of prehydrolyzed material, a coproduct of hydrolysis process, with respect to its higher rate and higher potential of delignification, is preferred to that of crude biomass. However, using prehydrolysis only for the purpose of delignification needs an economic assessment considering energy, time, and capital investment required for the process.

The results of the ANOVA for lignin and cellulose based on the factorial design are presented in Table 4. The values of *F* (Table 4) indicate that the simple (one-way) and the interactions of the factors including type of biomass, temperature, and residence time significantly ($\alpha = 1\%$) affected lignin and cellulose contents. The results of the regression modeling by the application of first-order polynomials for lignin mass fraction (in crude (y_{11}) and in prehydrolyzed

Table 4. ANOVA of Lignin and Cellulose as Dependent Variables in Delignification Process

	Lignin				Cellulose			
Source	df	SS	MS	*F*	df	SS	MS	*F*
Bio	1	1.0078	1.0078	968.92**	1	27576.24	27576.24	5533.36**
T	2	6.7359	3.3680	3238.03**	2	24187.28	12093.64	2426.67**
t	17	5.6201	0.3306	317.84**	17	19186.34	1128.61	226.46**
Bio × *T*	2	0.3415	0.1708	164.17**	2	3247.14	1623.57	325.78**
Bio × *t*	17	0.1384	0.0081	7.83**	17	1064.17	62.60	12.56**
T × *t*	34	0.6207	0.0183	17.55**	34	1903.67	55.99	11.23**
Bio × *T* × *t*	34	0.1013	0.0030	2.87**	34	616.80	18.14	3.64**
Error	216	0.2247	0.0010		216	1071.48	4.96	
Total	323	14.7904			323	78853.13		

Bio, type of the biomass (crude or prehydrolyzed); *T*, temperature; *t*, residence time; df, degree of freedom; SS, sum of squares; MS, mean of squares; *F*, *F* value in ANOVA; **, significant at 99% confidence level.

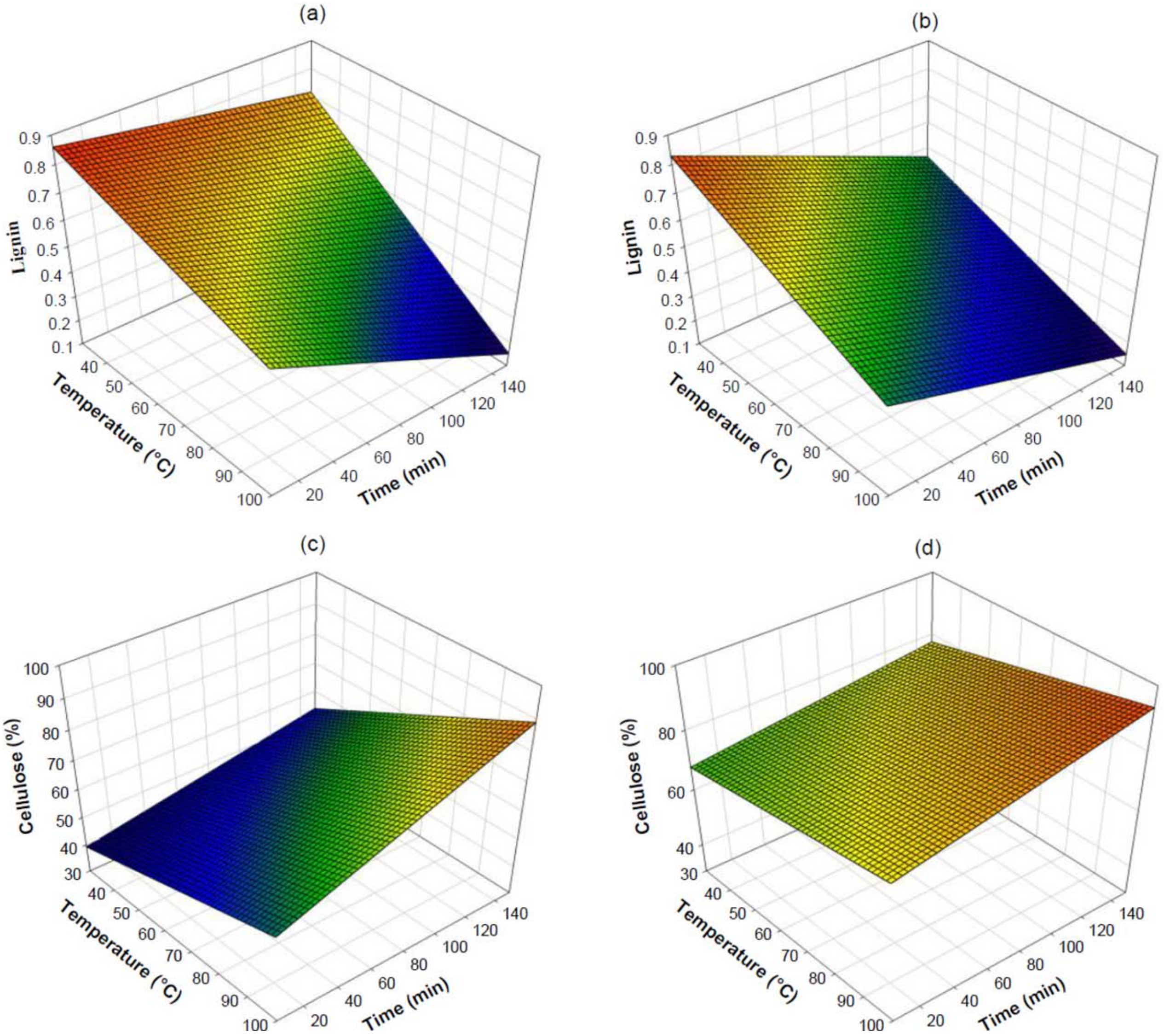

Figure 10. Response surface plots for the effects of residence time (5–150 min) and temperature (30–100°C) on: (a) lignin fraction in crude biomass, (b) lignin fraction in prehydrolyzed biomass, (c) cellulose fraction in crude biomass, and (d) cellulose fraction in prehydrolyzed biomass.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

(y_{12}) biomass) and cellulose percentage (in crude (y_{21}) and in prehydrolyzed (y_{22}) biomass) variations during delignification are presented in Eqs. 11–15.

Assuming: y_{11} and $y_{12} \leq 1.00$, y_{21} and $y_{22} \leq 100$, the residence time from 5 to 150 min, and the temperature in the range from 30 to 100°C, therefore

$$y_{11}=-0.00352\times(T)-0.00011\times(t)-0.00003\times(T\times t)+0.970,\ R^2=0.96 \tag{11}$$

$$y_{12}=-0.00523\times(T)-0.00268\times(t)+0.000005\times(T\times t)+0.998,\ R^2=0.91 \tag{12}$$

$$y_{21}=0.2936\times(T)+0.0577\times(t)+0.0014\times(T\times t)+29.93,\ R^2=0.97 \tag{13}$$

$$y_{22}=0.1414\times(T)+0.0480\times(t)+0.0006\times(T\times t)+63.13,\ R^2=0.99 \tag{14}$$

High values of coefficient of determination ($R^2 > 0.91$) were obtained with the models indicating that there is a very good fit of the polynomial models to the experimental results. The three-dimensional response surfaces, resulting from the aforementioned models, are shown in Figure 10. According to the response surfaces, a linear trend of gradient in the lignin and cellulose contents would exist with the factors of residence time (5–150 min) and temperature (30–100°C). This result agrees with the kinetic results in these ranges of residence time and temperature with the application of sodium hydroxide as the solvent. The polynomial models indicate that temperature and residence time have negative impact on lignin content but at the same time, they have positive impact on the cellulose content of both prehydrolyzed and crude biomass. Higher absolute values of the coefficients of temperature compared with those of the residence time indicate that temperature plays a more important role than residence time in the process of delignification and also the cellulose enhancement in the lignicellulosic biomass. The interactions of temperature and residence time are less important than the simple effect of the parameters due to the lower coefficients of the interactions presented in the polynomial models.

Conclusions

Hemicellulose removal of biomass in an acid-catalyzed hydrolysis process proved to be controlled by process parameters of temperature, catalyst (acid) concentration, and residence time. A combination of lower levels of temperature and catalyst concentration (110°C/0.1 N H_2SO_4) was not capable enough to remove all hemicellulose fractions from the biomass even at a residence time of 150 min. Therefore, higher concentration of catalyst was required to achieve 100% hemicellulose removal. An operation combination (temperature/catalyst concentration/residence time) of 130°C/0.1 N H_2SO_4/30–40 min was selected as the optimum condition for prehydrolysis of the material for the subsequent process of delignification. In the delignification process of intact or prehydrolyzed biomass, a biphasic sequential mechanism could express lignin dissolution very well compared with single-phase or three-phase mechanisms. Results indicated that prehydrolysis could be effective in accelerating as well as potential of lignin dissolution (removal). The kinetic data related to the activation energies of intact and prehydrolyzed materials showed that bulk stage required less energy of activation compared with the residual stage. This energy was almost the same (31–32 kJ/mol) for both materials in the bulk phase; however, it was less for the crude biomass in the residual phase. Overall, the full removal of hemicellulose in a hemicellulose-rich biomass can be considered as a dual-purpose approach in a biorefinery. The hemicellulosic hydrolysate can be used for xylan-based high-value products and at the same time, enhanced delignification (in terms of acceleration and potential) can be achieved for the cellulosic coproduct.

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