INFLUENCE OF ACID CONCENTRATION, TEMPERATURE, AND TIME ON DECRYSTALLIZATION IN TWO-STAGE CONCENTRATED SULFURIC ACID HYDROLYSIS OF PINEWOOD AND ASPENWOOD: A STATISTICAL APPROACH

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The effects on sugar yields of acid concentration, temperature, and time in the first (decrystallization) stage of a two-stage concentrated sulfuric acid hydrolysis of softwood (Scots pine) and hardwood (aspen) were investigated. The study focused on the multi-variable effects of the decrystallization stage and applied a statistical modeling with Central Composite Face (CCF) design of experiment to systematically study and simulate the effect of decrystallization reaction conditions on hydrolysis products and degradation products. The models were statistically significant and showed that for both aspen and pine, the reaction temperature and acid concentration were the most influential variables on monosaccharides and total sugar yields compared to the reaction time. The interaction between temperature and acid concentration was the most important for both species. The sugar degradation products were much influenced by the decrystallization temperature on both aspen and pine. The models were validated by a test-set and showed a good agreement between the experimental and predicted values. The optimum predicted total sugar yields were 56 g / 100 g d.w for aspen (74% theoretical) and 64 g / 100 g d.w for pine (91% theoretical).

Keywords: Concentrated acid Hydrolysis; Decrystallization; Lignocellulosic biomass; Bioethanol; Sulfuric acid

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INTRODUCTION

The utilization of lignocellulosic biomass as an alternative feedstock for production of bioethanol and other biofuels is gaining interest from a research perspective due to its high abundance, renewability, and low cost (Sun and Cheng 2002; Knauf and Moniruzzaman 2004; Galbe and Zacchi 2007). However, the recalcitrant nature of lignocellulosic biomass is a challenge to the hydrolytic breakdown of these materials to fermentable sugars (Himmel et al. 2007), thus limiting sugar yields and the overall biomass-to-ethanol process economics.

Unlike dilute acid hydrolysis, hydrolysis of lignocelluloses using concentrated acids achieves near-theoretical sugar yields with fewer degradation products (Schell and Duff 1996; Miller and Hester 2007a). The major drawbacks of this process include consumption of large quantities of concentrated acids, acid recovery constraints, high costs of neutralization, and gypsum disposal problems (Sherrard and Kressman 1945;

Sakai 1965; Hester and Farina 2000). However the invention of new acid recovery technologies and the high flexibility of this process towards different feedstocks including solid wastes have revived interest on this process, which was once left behind in research (Sheehan and Himmel 1999).

Efforts related to acid recovery started with the Hokkaido concentrated sulfuric acid process using diffusion dialysis with an ion exchange membrane to separate sugars from acid. In this process, 80% of the total sulfuric acid was recovered and the reconcentrated acid strength achieved 80% (Wenzl 1970). Research efforts on technologies of acid/sugar separation have continued since the interest in concentrated acid process was revived in the 1980s. Neuman *et al.* (1987) reported that nearly 100% sulfuric acid and 94% glucose could be recovered from acid/sugar solution samples containing 7.7 % H_2SO_4 and 1.0 % glucose by weight by ion exclusion chromatography. Nanguneri and Hester (1990) found that a cost decrease of up to 40% is achievable when using ion exclusion chromatography for sulfuric acid/sugar separation as compared to the traditionally used lime precipitation process. Springfield and Hester (1999) reported that 98% acid recovery could be achieved in a second exit stream containing 5 wt.% acid using a moving-bed ion exclusion chromatography system. Recently, successful acid recovery using organic solvents to extract the acid from the acid/sugar solution has been reported and patented (Weydahl 2010).

Concentrated sulfuric acid saccharification is a low-temperature high acid concentration process, which is carried out either in three stages (prehydrolysis, main hydrolysis/ decrystallization, and posthydrolysis) (Sakai 1965) or by a two-stage process in which biomass is mixed with concentrated acid to decrystallize cellulose and later hydrolyze the formed oligosaccharides with dilute acids to a mixture of hexoses and pentoses (Bayatmakooi et al. 1985; Liao et al. 2006). In both cases, decrystallization is an important stage, because during decrystallization the concentrated acids disrupt inter- and intramolecular hydrogen bonding responsible for cellulose crystallinity and render it amorphous and easily hydrolysable under mild conditions with formation of insignificant degradation products (Wright and Power 1985; Xiang et al. 2003). Thus, the proper control and management of reaction conditions is important to achieve an effective decrystallization and eventually higher sugar yields (Sherrard and Harris 1932; Hon and Shirashi 1991).

Degradation of sugars during hydrolysis is a critical problem for acid processes because sugar degradation not only lowers the conversion yields, but also generates fermentation inhibitors that can inhibit the fermenting microorganisms during the downward fermentation process, thus jeopardizing sugar to ethanol conversion yield and the overall biomass-to-ethanol conversion efficiency (Taherzadeh et al. 1997; Larsson et al. 1998; Sanchez et al. 2004). During hydrolysis in acidic medium, pentoses degrade to furfural, while hexoses degrade to 5-hydroxymethyl furfural (HMF). Further degradation of furfural leads to the formation of formic acid, while HMF degrades further to levulinic acid and formic acid (McKibbins et al.1962). Acetic acid is liberated from the side groups of hemicelluloses during hydrolysis. The reaction products can be recovered from hydrolyzates by different technologies such as adsorption on activated carbon, or hydrophobic zeolites and can be utilized as base chemicals for production of alternative fuels, drugs, and polymeric materials (Ranjan et al. 2009). However, given the

comparatively lower production of fermentation inhibitors in the concentrated acid process, removal of fermentation inhibitors simply for detoxification purposes seems more appropriate. Overlimining (pH 9-10) with $Ca(OH)_2$ is the widely used technology (Martinez et al. 2001). Other investigated detoxification methods include ion exchange resins (Nilvebrant et al. 2001) or enzymatic detoxification (Jönsson et al. 1998).

Although the influence of parameters affecting lignocellulose decrystallization and hydrolysis has been studied (Goldstein et al. 1983; Bayat-makooi et al. 1985; Bayat-Makooi and Goldstein 1985; Kunihisa and Ogawa 1985; Camacho et al. 1996; Xiang et al. 2003; Miller and Hester 2007a), focus has been on a one-variable-at-a-time (OVAT) approach. To the authors' knowledge, there are a limited amount of studies which have embarked on a systematic multi-variable analysis of the decrystallization stage, thus limiting the amount of data and understanding of combined effects, interactions, and synergies or antagonism of the process variables during decrystallization.

This study aimed to investigate systematically the individual and synergistic (interactions) effects of the major operating variables (reaction temperature, acid concentration, and residence time) at the decrystallization stage and their influence on the sugar recovery and subsequent sugar degradation. This was accomplished by employing statistical modeling with design of experiment (DOE) to correlate the effect of decrystallization reaction conditions (independent variables) on sugar yields (dependent variables or responses) in a two-stage concentrated sulfuric acid hydrolysis of softwood (Scots pine) and hardwood (aspen). The developed empirical models were further validated by a test-set.

EXPERIMENTAL

Raw Materials (Substrates)

The biomass types considered in this study were the Nordic wood species trembling aspen (*Populus tremula*) and Scots pine (*Pinus sylvestris*). The supplied wood chips were dry. The wood chips were milled in a hammer mill and screened, and the fraction between 3mm and 7mm was retained for decrystallization experiments. The retained fraction was analytically screened in a laboratory sieve shaker, and the size distributions are shown in Table 1.

Table 1. Size Distribution of Aspen and Pine Wood Chips

Sieve Size (mm)	Percent by mass retained (%)	
	Aspen	Pine
>5.6	0.0	2.2
4.0 - 5.6	12.0	16.2
2.8 - 4.0	42.0	25.6
1.7 - 2.8	35.3	41.4
1.2 - 1.7	8.1	12.5
<1.2	2.7	2.2

Average size for both aspen and pine is 2.9 mm

Biomass Composition Analysis

The analysis of the chemical composition of the biomass used in this study was based on analytical procedures developed by the National Renewable Energy Laboratory (NREL). The biomass was prepared according to Hames et al. (2008). In summary, the wood chips were milled with a laboratory mill machine (Anthony H. Thomas Co., Philadelphia, USA) and sieved. The fraction below 150 μm was used for determination of ash content, while the fraction between 150 and 841 μm was used for determination of extractives, lignin, and carbohydrates. The dry matter content was analyzed by a halogen moisture analyzer (Mettler Toledo, HR73). Ash content was analyzed according to the method by Sluiter et al. (2008a) in a muffle furnace at 575°C for 20 hours. Ethanol extractives were determined according to Sluiter et al. (2008c) by a 22 hours Soxhlet extraction, and the solvent was removed by the Turbo VapII method.

Structural carbohydrates and lignin analyses were performed in a two-stage acid hydrolysis procedure according to Sluiter et al. (2008b). Briefly, the extractive-free samples were soaked in 72 wt.% sulfuric acid at 30 °C in a water bath and stirred regularly for 1 hour. The decrystallized sample was then hydrolyzed at 4 wt.% sulfuric acid at 121 °C in an autoclave for 1 hour. The solid residues were measured gravimetrically for acid insoluble lignin after oven drying at 105°C overnight while the liquid fraction was analyzed for monosaccharide composition by HPLC, and acid soluble lignin by spectrophotometry at 320 nm. Table 3 shows the chemical composition of aspen and pine wood samples used in this study.

Decrystallization and Hydrolysis for Saccharification

Decrystallization

The saccharification was performed in two stages, the decrystallization stage and the hydrolysis stage. In the decrystallization stage, 3.0 g oven dry weight (o.d.w) of pre-steamed wood chips was gradually mixed with a predetermined volume of deionized water and 96 to 98 wt. % of sulfuric acid in a 250-mL Pyrex Erlenmeyer flask with head stopper to obtain the required acid concentration. The mixture was put in a shaking water bath (Stuart Scientific SBS 30) preset at the required reaction temperature and a shaking speed of 200 rev/min to provide adequate mixing of the biomass. All experiments in the decrystallization stage were performed at a liquid-to-wood ratio or liquor-to-solid ratio of 15 (w/w) to ensure complete biomass wetting and minimize diffusion controlled decrystallization due to mixing limitations.

Hydrolysis

After decrystallization, the black paste of decrystallized wood material was diluted with deionized water to an acid concentration of 20 wt. % in a 250 mL plastic capped Pyrex glass bottle and hydrolyzed at 100 °C in an autoclave (certoclav, CV-EL 12 L GS) open to the atmosphere for 3 hours to complete the hydrolysis. The slurry was cooled in an ice bath to stop the reaction and then filtered to separate the solid lignin from the acid/sugar solution by vacuum filtration. The solid residue was washed three to four times and dried in an oven at 105 °C overnight to determine the gravimetric solid residue. The acid/sugar solution was then neutralized with solid calcium hydroxide to between pH 5.0 and 6.0, and the solution was further filtered to separate the precipitating $CaSO_4$

(gypsum) from a yellowish sugar solution. The sugar solution was stored in a refrigerator at 4°C before monosaccharide and degradation products analysis by HPLC. To assess the decrystallization effect, the hydrolysis was standardized at the same reaction conditions for all samples.

The individual monosugars and degradation products yields were calculated based on the original dry wood as shown in Equation 1,

$$Y = \frac{x \cdot V}{W} \cdot 100 \quad (1)$$

where Y is monosugar or degradation product yield in g/100 g dry wood; x is the concentration of the sugar component or degradation product in the liquid phase (g/L); V is the total volume of the liquid phase in decrystallization and hydrolysis (L), and W is the dry weight of original woods sample (g). The total sugar yield expressed as grams total sugar per 100 g of original o.d.w was calculated as the sum of the yields of all monosaccharides.

Experimental Design and Data Analysis

A response surface methodology (RSM) technique using MODDE 8.0.2 software (Umetrics AB (Umeå, Sweden)) was used to systematically investigate the effects of the three independent variables (temperature, acid concentration and time) on sugar yields and degradation (dependent/response variables) during decrystallization. RSM also optimizes the responses. The two levels and three factors Central Composite Face (CCF) design of experiment employed in this study consisted of a total of 17 ($2^k + 2k + n_c$) experiments with 8 runs at the corner or cube points (2^k), 6 axial points ($2k$), and 3 repeats at the centre point (n_c), where k is the number of factors and n_c is the number of repeats at the centre point. It is worth noting that the CCF design of experiment was applied only to the decrystallization stage of the process. Table 2 shows the coded and actual levels of the independent variables.

Table 2. Experimental Design for the Decrystallization Stage, Coded and Actual Factor Levels

Variables	Coded levels		
	Low -1	Middle 0	High 1
Temperature, θ (^{o}C)	35	52.5	70
Reaction time, T (min)	60	120	180
Acid concentration, C (wt.%)	65	72.5	80

The upper and lower limits of the conditions shown in Table 2 were selected based on the reported information in literature (Sherrard and Kressman 1945; Harris 1949; Goldstein 1980; Bayat-makooi et al. 1985; Hon and Shirashi 1991; Camacho et al. 1996; Xiang et al. 2003).

The experimental data were fitted to the second-order regression model in

equation 2 to correlate the response parameters (sugar yields and degradation products) as a function of temperature, acid concentration and time (factors) at the decrystallization stage.

$$Y_{SY} = \Psi_0 + \Psi_1\theta + \Psi_2 T + \Psi_3 C + \Psi_{12}\theta^* T + \Psi_{13}\theta^* C + \Psi_{23} T^* C + \Psi_{11}\theta^2 + \Psi_{22}T^2 + \Psi_{33} C^2 + \ldots + \varepsilon \quad (2)$$

In Eq. 2 Y_{SY} is a response (dependent) parameter under investigation, in this case monosaccharide yield, total sugar yield or degradation products (g/100 g d.w), whereas θ, T, and C are coded values of reaction temperature (^oC), reaction time (minutes), and sulfuric acid concentration (wt.%) respectively. Ψ_i represents regression coefficients for the linear term effect, Ψij for the interaction term effect, Ψ_{ii} for the quadratic term effect, Ψ_0 is the constant (interception coefficient) term, and ε is the random error, assumed to be normally distributed.

Coding of the actual variables levels for statistical regression analysis is shown in Equation 3,

$$z_i = (Z_i - Z_m) / \Delta Z \quad (3)$$

where z_i is the coded value of the variable, Z_i is the variable's actual value, Z_m is the middle value of the variables, and ΔZ is the step change of a variable (Wen and Chen 2001).

Multiple Linear Regression (MLR) included in MODDE 8.0.2 software was used for statistically fitting the second-order model in Equation 2 to the experimental data.

Analytical Methods

HPLC analysis of monomeric sugars and sugar degradation products in hydrolyzates

The HPLC system used in this work was from Shimadzu (Kyoto, Japan) consisting of an LC-10AD pump, a Rheodyne 20 μL manual injector, CTO-10A column oven, RID-6A refractive index detector, SPD-6A ultraviolet (UV-VIS) spectrophotometer detector, CMB-20A controller, and Shimadzu's LC-Solution software (release 1.25) for acquiring the chromatograms.

Analysis of monosaccharide composition in hydrolyzates for glucose, xylose, galactose, arabinose, and mannose was performed on a Chrompack Carbohydrates Pb column (Varian, Palo Alto, CA, USA) with deionized and degassed water as mobile phase and RI detection. Column temperature was 80 °C, and the flow rate was 0.4 mL/min. The analytical column was in line with a cation (H^+)/anion (CO_3^-) deashing guard column (Biorad 125-0118). Mannitol was used as internal standard in the sugar analysis.

Quantification of sugar degradation products in hydrolyzates was performed using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) in line with a Cation-H guard column (Biorad 125-0129) and ultraviolet (UV-VIS) absorbance detection. Mobile phase was 5 mM sulfuric acid. Flow rate was 0.6 mL/min, and column temperature was 65 ^oC. 5-hydroxymethylfurfural (HMF), furfural, and levulinic acid were detected at 280

nm, while acetic acid and formic acid were detected at 210 nm .

All hydrolyzate samples were filtered through a 0.2 μm filter before injection into the HPLC.

Estimation of peak area of chromatograms

Lack of baseline separation and peak overlapping is commonly observed on some sugar columns, especially between the glucose and xylose peaks, and between the arabinose and mannose peaks. This may introduce an error during peak area estimation with chromatography software. To reduce this possible error, the sugar chromatograms were exported as ASCII (American Standard Code for Information Interchange) files and reprocessed by Peakfit® software version 4 from SPSS Inc (Vaaler et al. 2001). The Gaussian deconvolution method built in the Peakfit® commercial software for chromatographic data was used for refitting of the chromatograms to the required number of individual peaks (Goodness of fit at coefficient of determination $R^2 = 0.99$).

RESULTS AND DISCUSSION

Chemical Composition of Raw Materials

The chemical compositions of aspen and Scots pine wood are shown in Table 3.

Table 3. Composition of aspen and scots pine feedstocks (wt. % on o.d.w. of original wood)

	Aspen		Pine	
	Values	Totals	Values	Totals
Glucan	45.6		43.6	
Mannan	1.8		11.3	
Galactan	1.7		1.5	
Hexosans		49.1		56.4
Xylan	17.9		6.4	
Arabinan	0.5		0.9	
Pentosans		18.4		7.3
(Carbohydrate polymers)		(67.5)		(63.7)
Acid insoluble lignin	18.6		26.1	
Acid soluble lignin	0.6		0.3	
Total lignin		19.2		26.4
Extractives	3.1		2.3	
Ash	0.5		0.5	
Low molecular mass compounds		3.6		2.8
Unaccounted*	9.7	9.7	7.1	7.1
Total	100.0	100.0	100.0	100.0

* Based on literature data, most of the material unaccounted for is believed to be uronic acids and acetyl content in hemicelluloses.

It can be deduced that the maximum potential/ theoretical glucose yield for aspen and pine is 50.7 and 48.4 g/100 g dry wood, respectively, while the maximum potential/ theoretical total sugar yields is 75.5 and 70.3 g total sugars/100 g dry wood for aspen and pine, respectively. Note the increase in mass by addition of one molecule of water during the hydrolysis of hexosans to hexoses (18/162 or 11.11%) and pentosans to pentoses (18/132 or 13.64%).

The variability in chemical composition and structure between softwoods and hardwoods has been reported to have a significant impact of the conditions required for processing these feedstocks. Overend and Chornet (1987) reported that softwoods are less easily treated as compared to hardwoods and usually need a combined chemical such as SO_2 or H_2SO_4 and steam-aqueous treatment for fractionation. According to Niemzl et al. (2010), the dominance of a thermally stable glucomannan backbone in softwood hemicelluloses as compared to the glucuronoxylan or simply xylan backbone in hardwood hemicelluloses influences the hemicelluloses solubilization. The presence of higher amounts of condensed lignin in softwoods may hinder the swelling of the cell wall during hydrolysis (Phaiboonsilpa et al. 2009). The high hydrolysis recalcitrance of softwoods may also be attributed to the higher cellulose crystallinity of softwoods as compared to hardwoods (Newman 1994), since crystallinity has been reported to affect cellulose accessibility by acids (Zhao et al. 2006).

Decrystallization and Hydrolysis

The measured monosugar concentrations of the typical hydrolyzates from the two-stage concentrated sulfuric acid hydrolysis process and their corresponding yields are shown in Table 4. The term hydrolyzate is repeatedly used to represent the sugar solution produced after hydrolysis and neutralization.

Table 4. Typical Decrystallization Conditions and Measured Monosugar Concentrations and Yields of Hydrolyzates Derived from a Two Stage-Concentrated Sulfuric Acid Process for Aspen and Pine

Biomass	Decrystallization conditions			Monosugar concentrations (g/L)					Monosugar yields (g/100 d.w)				
	Temp (°C)	Time (min)	Acid conc (wt.%)	Glc	Xyl	Gal	Ara	Man	Glc.	Xyl	Gal	Ara	Man
Aspen	35	180	65	7.01	2.62	0.46	0.13	0.36	35.76	13.36	2.37	0.66	1.85
Pine	52.5	60	72.5	7.73	0.64	0.35	0.25	1.65	44.00	3.64	1.98	1.40	9.40

Key: Temp-Temperature; Acid conc-Acid concentration; Glc-Glucose; Xyl-Xylose; Gal-Galactose; Ara-Arabinose; Man-Mannose

Although the acid-to-wood ratio at decrystallization should be kept as low as 1:1 for economic benefits of the process (Wenzl, 1970), such a low acid-to-wood ratio can be achieved in practice by the use of efficient mixing and shearing equipment such as a twin-screw extruder reactor (Miller and Hester 2007). However, achieving such a low acid-to-wood ratio in this study would be a challenge due to mixing limitations as

explained earlier and the relatively large particle size.

Although the second stage/hydrolysis conditions were fixed in order to evaluate the decrystallization effect on sugar yield, it is worth noting that the hydrolysis conditions may affect the sugar recovery. A prolonged hydrolysis of 3 hours used in this study aimed to ensure that all the polymeric sugars are fully converted to monosugars. The absence of oligomers in the hydrolyzates (Fig. 1) demonstrated that the hydrolysis conditions used in this study completely converted the polymeric sugars to monosugars. Clausen and Gaddy (1993) showed that hydrolysis conditions of 29 and 31 wt.% sulfuric acid at 100 ^{o}C required about 20 minutes for total conversion of polymeric sugars to monosugars in the concentrated sulfuric acid pretreated corn stover. They also showed that the time for total monomer conversion varied at diffent temperatures and acid concentrations, however a partial hydrolysis was observed at 70^{o}C and 30-40 wt.% for 60 minutes. Investigating the hydrolysis of decrystallized biomass at higher temperatures, Miller and Hester (2007b) showed that hydrolysis of pretreated (decrystallized) pine sawdust at 30 wt. % sulfuric acid and 130^{o}C required about 3 minutes to convert 41% of the theoretical glucose, while diluting the acid down to 5 wt.% at the same temperature increased the hydrolysis time to 25 minutes for 50% theoretical glucose conversion.

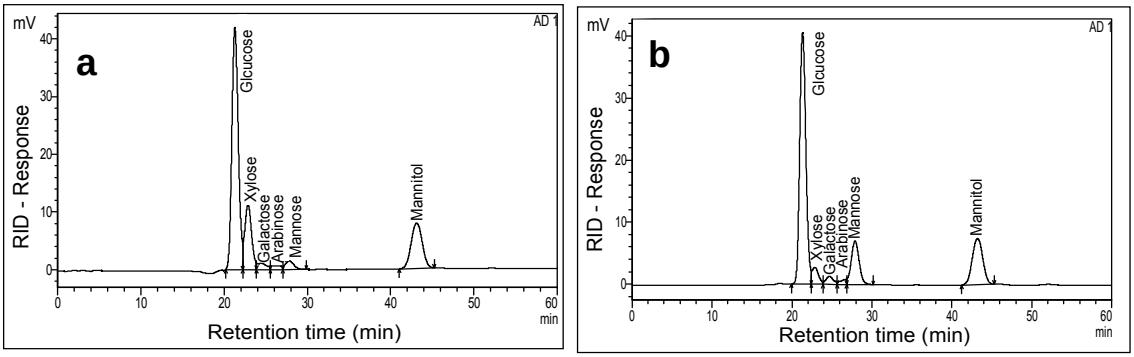

Fig. 1. Typical HPLC chromatogram profile for hydrolyzate obtained from a two-stage concentrated sulfuric acid process and separated on Chrompack Carbohydrates Pb column. Mannitol used as an internal standard. (a) aspen, (b) pine. Decrystallization conditions, measured monosugar concentrations and corresponding yields for these hydrolyzates are shown in Table 4.

Statistical Modeling of the Decrystallization Stage

The results from the 17 initial experiments from the CCF design in Table 2 were first statistically regressed in MODDE® 8.0.2. After a preliminary investigation of the contour plots, 10 additional experiments were selected and added to explore the region at lower temperatures of up to 20 ^{o}C outside the original design matrix. It is worth noting that the modeling results reported in this study were based on a total of 27 experiments for aspen or pine.

A logarithmic transformation was performed on the aspen response values to improve the fitting. No transformation was taken on the pine responses. A second-order model equation (Eq. 2) was fitted to the experimental yields and regressed by MLR to simulate the influence of reaction temperature, acid concentration, and reaction time on monosaccharide, total sugar yields and sugar degradation products on both aspen and

pine.

Investigation of normal probability plots of residuals showed that experiment number 21 (20 ^{o}C, 180 min, 80 wt.%) for pine and experiment run number 16 (70^{o}C, 180 min, 80 wt.%) for aspen had large absolute values of studentized residuals and hence were considered as outliers and excluded from the modeling. The randomized deleted studentized residuals for the predicted values did not show any patterns for either the pine or the aspen models. The statistical significance of coefficients of the model terms was evaluated at 95% confidence level (p-value < 5%). The analysis of variance (ANOVA) was used to statistically evaluate the significance of the individual empirical models.

Evaluation of the Developed Models

Tables 5 and 6 show the regression coefficients of the models and their corresponding statistical parameters for goodness of fit. The model coefficients for each response were obtained by fitting the experimental data to the second-order model in equation 2 by MLR.

Table 5. Aspen Regression Coefficients and p-values for Selected Responses (Coefficients estimated at 95% confidence level) [a]

Coeff. Symb.	Variable[b]	Glucose		Xylose		Mannose		Total sugar	
		Coeff. SC	p-value	Coeff. SC	p-value	Coeff. SC	p-value	Coeff. SC	p-value
Ψ_0	Constant	1.42	0.00	0.57	0.00	-0.02	0.83*	1.54	0.00
Ψ_1	Temp	-0.26	0.00	-0.45	0.00	-0.17	0.04	-0.24	0.00
Ψ_2	Tim	0.02	0.59*	-0.11	0.05*	-0.05	0.53*	-0.01	0.56*
Ψ_3	Conc	-0.26	0.00	-0.52	0.00	-0.27	0.00	-0.23	0.00
Ψ_{11}	Temp*Temp	-0.11	0.00	-0.17	0.00	-0.08	0.16*	-0.10	0.00
Ψ_{22}	Tim*Tim	-0.05	0.18*	0.03	0.63*	-0.08	0.50*	-0.05	0.16*
Ψ_{33}	Conc*Conc	-0.10	0.02	-0.09	0.22*	-0.08	0.54*	-0.06	0.09*
Ψ_{12}	Temp*Tim	0.00	0.93*	-0.04	0.29*	0.02	0.77*	-0.01	0.57*
Ψ_{13}	Temp*Conc	-0.12	0.00	-0.14	0.00	-0.01	0.90*	-0.08	0.00
Ψ_{23}	Tim*Conc	0.01	0.65*	-0.01	0.80*	-0.07	0.34*	0.00	0.96*
Goodness of fit									
R^2		0.94		0.94		0.64		0.95	
Q^2		0.67		0.72		-0.56		0.75	

[a] aspen coefficients are base 10 logarithmically transformed

[b] Temp=Temperature; Conc=Acid concentration; Tim= Time; Coeff. SC=Scaled and centred coefficient.

* Not significant term

Table 6. Pine Regression Coefficients and p-values for Selected Responses (Coefficients estimated at 95% confidence level)

Coeff. Symb.	Variable[a]	Glucose		Xylose		Mannose		Total sugar	
		Coeff. SC	p-value	Coeff. SC	p-value	Coeff. SC	p-value	Coeff. SC	p-value
Ψ_0	Constant	38.30	0.00	3.38	0.00	7.50	0.00	52.25	0.00
Ψ_1	Temp	-11.37	0.00	-1.73	0.00	-3.01	0.00	-16.63	0.00
Ψ_2	Tim	-0.92	0.39*	-0.43	0.31*	-0.86	0.05	-2.48	0.12*
Ψ_3	Conc	-7.16	0.00	-1.11	0.01	-2.83	0.00	-11.33	0.00
Ψ_{11}	Temp*Temp	-5.97	0.00	0.05	0.88*	-1.03	0.01	-7.18	0.00
Ψ_{22}	Tim*Tim	3.27	0.09	-1.20	0.11*	0.32	0.64*	2.00	0.46*
Ψ_{33}	Conc*Conc	-11.40	0.00	-0.08	0.92*	-0.97	0.19*	-12.60	0.00
Ψ_{12}	Temp*Tim	-0.15	0.87*	0.02	0.96*	-0.23	0.52*	-0.62	0.65*
Ψ_{13}	Temp*Conc	-5.44	0.00	-0.02	0.97*	-0.65	0.08	-6.27	0.00
Ψ_{23}	Tim*Conc	-2.46	0.04	-0.38	0.40*	-0.25	0.56*	-3.19	0.07
Goodness of fit									
R^2		0.95		0.81		0.90		0.94	
Q^2		0.73		0.38		0.45		0.70	

[a] Temp=Temperature; Conc=Acid concentration; Tim= Time; Coeff. SC=Scaled and centred coefficient.
* Not significant term

The regression correlation coefficient values of percent variation of the response explained by the model (R^2) and percent variation of the response predicted by the model according to cross validation (Q^2) for monosaccharides and total sugar empirical models in aspen (Table 5) and pine (Table 6) were good enough ($R^2 > 0.8$ and $Q^2 > 0.4$). Exclusion of the insignificant terms further improved the regression coefficients of the models (Table 7).

Table 7 shows the reduced empirical model equations with only significant terms and coefficients ($p < 0.05$) after excluding the insignificant terms. The R^2 and Q^2 values in Table 7 show that the fitting of the second-order model in eqn. (2) to the experimental data was adequate and there was a good agreement between the experimental sugar yields and those predicted from the models. The R^2 values also show that there was a good correlation between the operational variables (temperature, acid concentration, and time) and the responses (monosaccharides and total sugar yields). The Q^2 values in Table 7 show that the models have a good predictive power and anticipated small errors of prediction ($Q^2 > 0.7$) with the exception of mannose in aspen and xylose in pine. The low Q^2 values of mannose in aspen and xylose in pine is possibly due their low contents in the

respective substrates, hence resulting into erroneous yield estimation. The ANOVA test at 95% confidence level in Table 7 shows that the monosaccharide and total sugar models for aspen and pine were highly significant. Figure 2 compares graphically the experimental total sugar yields and those predicted from the models in Table 7.

Table 7. The Reduced Sugar Yields Predictive Empirical Models as a Function of Coded Operating Variables (coefficients evaluated at 95% confidence level)

Response	Model equation[a]	R^2	Q^2	ANOVA Test RSD*sqrt(F(crit))< SD Regression[b]	
				RSD* sqrt(F(crit))	SD Regn
	Aspen[c]				
Glucose	$Y = 1.42-0.27\cdot\theta -0.27\cdot C-0.12\cdot\theta^2 -0.13\cdot C^2 - 0.13\cdot \theta\cdot C$	0.93	0.77	0.12	0.49
Xylose	$Y = 0.57-0.43\cdot\theta -0.50\cdot C -0.16\cdot\theta^2 -0.13\cdot \theta\cdot C$	0.93	0.82	0.22	0.87
Mannose	$Y = -0.17\cdot\theta -0.27\cdot C$	0.61	0.05	0.36	0.50
Total sugar	$Y = 1.53-0.24\cdot\theta -0.23\cdot C -0.11\cdot\theta^2 -0.08\cdot C^2 - 0.07\cdot \theta\cdot C$	0.94	0.82	0.10	0.44
	Pine				
Glucose	$Y = 38.78-11.00\cdot\theta -7.00\cdot C-5.62\cdot\theta^2 -9.65\cdot C^2 -5.50\cdot\theta\cdot C-2.55\cdot T\cdot C$	0.93	0.83	5.78	21.57
Xylose	$Y = 3.20-1.86\cdot\theta -1.18\cdot C$	0.77	0.48	2.28	4.18
Mannose	$Y = 7.56-2.96\cdot\theta -2.83\cdot C-1.02\cdot\theta^2$	0.90	0.69	2.07	6.27
Total sugar	$Y = 52.59-16.37\cdot\theta - 11.27\cdot C-7.04\cdot\theta^2 - 11.55\cdot C^2 -6.21\cdot\theta\cdot C-3.37\cdot T\cdot C$	0.94	0.83	7.98	31.28

[a] θ, T, and C are coded values of reaction temperature (°C), reaction time (minutes), and sulfuric acid concentration (wt.%) respectively.

[b] SD Regression (SD Regn): Standard Deviation is the square root of MS (mean square) regression; RDS: Residuals standard deviation; The critical F is the value of the F-distribution over which SD regression is statistically significant at the 95% confidence level. When RSD*sqrt(F(crit)) is smaller than SD Regression, the model is significant at 95% confidence level (Umetrics AB (Umeå, Sweden).

[c] aspen coefficients are base 10 logarithmically transformed

Y= yield (g /100 g d.w)

Effect of Process Variables on Sugar Yields

The effects of process variables and their interaction on sugar yields can be interpreted from their corresponding coefficients values. In MODDE® 8.0.2, the values of the effects are twice the coefficients (Umetrics AB, Umeå, Sweden). The same effects were visualized graphically from the effects plots.

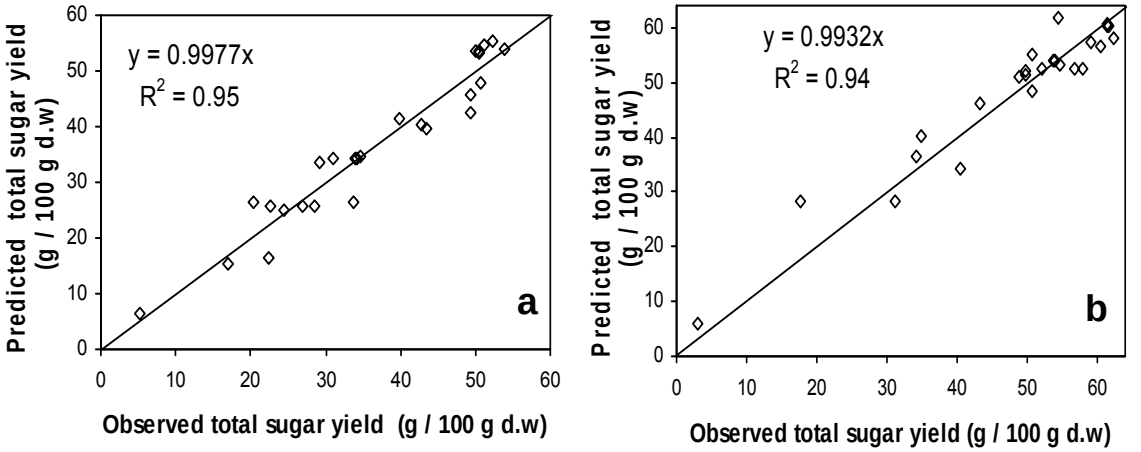

Fig. 2. Correlation between experimental and predicted total sugar yields (a) aspen (b) pine

Table 7 shows that the acid concentration and the reaction temperature were statistically the most influential process variables in both aspen and pine decrystallization. For aspen, the acid concentration and the reaction temperature had almost the same magnitude of influence for the investigated responses. The reaction time was the less important process variable. Table 7 also shows that the temperature/acid concentration was the most significant interaction in aspen decrystallization for all responses except for mannose which showed linear effects only. The high influence of acid concentration on wood and cellulose hydrolysis has been reported by Hon and Shirashi (1991). Clausen and Gaddy (1993) have also shown that temperature has a high influence on conversion of biomass to monosugars.

For pine, the reaction temperature had more influence on decrystallization, with the effect's magnitude being almost 1.5 times higher than the acid concentration for all responses except for mannose yield, where both temperature and acid concentration had almost equal effect (Table 7). The importance of temperature in concentrated acid hydrolysis has been reported (Sherrard and Harris 1932). The temperature/acid concentration interaction was also the most important interaction for pine except in xylose and mannose where the interaction was insignificant. The reaction time remained to be the statistically less important process variable for pine as well.

The variability of sugar yields (responses) as a function of operating variables when varied from low to high levels can be studied by the 3D surface response and 2D-contour plots. Figure 3 depicts the influence of temperature and acid concentration on total sugar yield in aspen and pine on a contour plot at low level of the reaction time. The high degree of curvature in Figure 3a shows that both temperature and acid concentration had strong quadratic effects on total sugar yield in aspen. Increasing both temperature and acid concentration also increased the total sugar yield and the maximum yield of about 56 g sugars/100 g dry wood occurred in the ranges of 30 to 40 ^{o}C and 65 to 69 wt.% for the investigated range; however it is worth noting that the acid concentration for highest total sugar yield in aspen seem to be just below 65 wt.% (Fig. 3a). Further increase in both temperature and acid concentration decreased the total sugar yield.

Figure 3b shows total sugar yield as a function of temperature and acid concentration for pine. The strong quadratic effect of temperature and acid concentration on total

sugar yield of pine was apparently significant. It can also be seen that the total sugar yield increased with increasing temperature and acid concentration and the maximum yield of about 64 g sugars/100 g dry wood appears in the temperature range of 28 to 40°C and acid concentration between 70 and 74 wt.%. Further increase in temperature and acid concentration decreased the total sugar yield.

The high significance of the main effect (linear), interaction, and quadratic effects shown by the reaction temperatures and acid concentration indicate that these variables had more impact in the decrystallization of both aspen and pine. The temperature-acid concentration appeared to be the most important among other interactions in both aspen and pine because of the high influence revealed by these variables (Table 5 and 6).

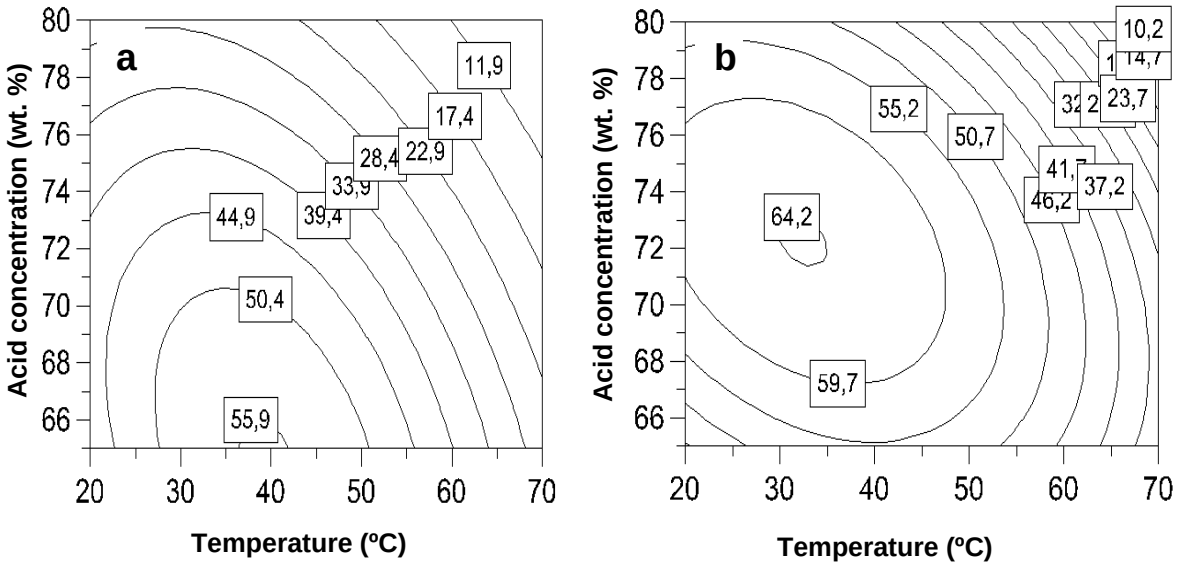

Fig. 3. Constant yield contour plots showing the effect of temperature and acid concentration on the total sugar yield at decrystallization time of 60 minutes (a) aspen (b) pine

Although the acid concentration and reaction temperature independently have shown high influences on hydrolysis, Hon and Shirashi (1991) reported that a much better conversion efficiency could be obtain at a moderate acid concentration (62 %) when the temperature was raised to 40 °C as compared to hydrolysis yield at 80 % and 0 °C. This observation agrees with what was found in this study with respect to the synergistic effect of temperature and acid concentration apparently revealed by the strong interaction terms between the two variables for both aspen and pine (Table 7 and Fig. 3).

The constant total sugar yield contours in Figure 3 show an inverse relationship between temperature and acid concentration for both aspen and pine. This implies that decrystallization at high acid concentration requires low temperature to achieve high sugar yields. The temperature-acid concentration inverse correlation has also been observed for dilute acid hydrolysis at high temperature (Neureiter et al. 2002).

Effect of Decrystallization Conditions on Sugar Degradation Products

The correlation between decrystallization conditions and monosugar dehydration to HMF and furfural can be deduced from the model coefficients in Table 8. It can be

seen that the reaction temperature had more influence on the formation of furfural and HMF for both aspen and pine. The HMF and furfural yields increased with increase in glucose and xylose yields respectively. This was anticipated because the sugar degradation reactions in the reactor depend on the sugar concentration as their initial substrate concentration.

The yield of HMF and furfural in pine ranged from 0.01 to 0.27 g/100g d.w and 0.01 to 0.88 g/100g d.w, respectively, with the highest concentrations being 49.90 mg/L and 172.96 mg/L, respectively. For aspen, the yield of HMF and furfural ranged from 0.07 to 0.19 g/100g d.w and 0.02 to 2.30 g/100g d.w, respectively, corresponding to maximum concentrations of 37.59 mg/L and 451.10 mg/L, respectively. The HMF and furfural concentration values are slightly higher than those reported by Heinonen and Sainio (2010) from spruce and birch due to mild hydrolysis at 80^{o}C. The relatively high yield of furfural is due to the vulnerability of xylose degradation in acid medium and the relatively high stability of furfural toward further degradation, while the low yield of HMF is attributed to the high stability of glucose and the instability of HMF towards further degradation. The rate of sugar degradations under acidic medium follows the order of Xylose > Arabinose > Mannose > Galactose > Glucose (Taherzadeh and Karimi 2007). The detected levels of formic acid of up to 6.77 g/L and 2.71 g/L and levulinic acid up to 1.86 g/L and 1.63 g/L in pine and aspen hydrolyzates, respectively, indicates a significant further degradation of furfurals and HMF possibly caused by the relatively high acid concentration of 20 wt.% during hydrolysis. The highest levels of acetic acid in pine and aspen were 3.54 g/L and 1.94 g/L, respectively.

Table 8. The Reduced Predictive Empirical Models for Yield of Sugar Degradation Products as a Function of Operating Variables (coefficients evaluated at 95% confidence level; variables are coded)

Response	Model equation	R^2	Q^2	ANOVA Test RSD*sqrt(F(crit))< SD Regression[a]	
				RSD* sqrt(F(crit))	SD Regn
	Aspen[b]				
HMF	$Y=-0.83 -0.11\cdot\theta-0.06\cdot C-0.06\cdot\theta^2$	0.77	0.54	0.09	0.18
Furfural	$Y=-0.43 -0.60\cdot\theta - 0.16\cdot T - 0.57\cdot C- 0.18\cdot\theta^2 - 0.25\cdot \theta\cdot C$	0.92	0.79	0.30	1.10
	Pine				
HMF	$Y = 0.22-0.06\cdot\theta -0.03\cdot C-0.03\cdot\theta^2 -0.04\cdot C^2 - 0.03\cdot\theta\cdot C-0.02\cdot T\cdot C$	0.86	0.70	0.04	0.11
Furfural	$Y = 0.33-0.20\cdot\theta -0.12\cdot C$	0.74	0.42	0.31	0.52

[b]aspen coefficients are base 10 logarithmically transformed

Validation of the Sugar Prediction Models

Although cross-validation is carried out during the mathematical model building using the calibration data set in different data combinations, i.e. full, random, custom, or systematic cross validation, a test-set validation takes the model closer to reality. One-variable-at-a-time validations of the sugar models were done by running independent experiments with conditions within the investigated range but different from those used for modeling. Figures 4 and 5 compare the total sugar yield predicted from the models in Table 7 with the total sugar yield determined experimentally (test-set). The close coincidental trends between the predicted and experimental total sugar yields confirm the validity of the models within the investigated range and within experimental error.

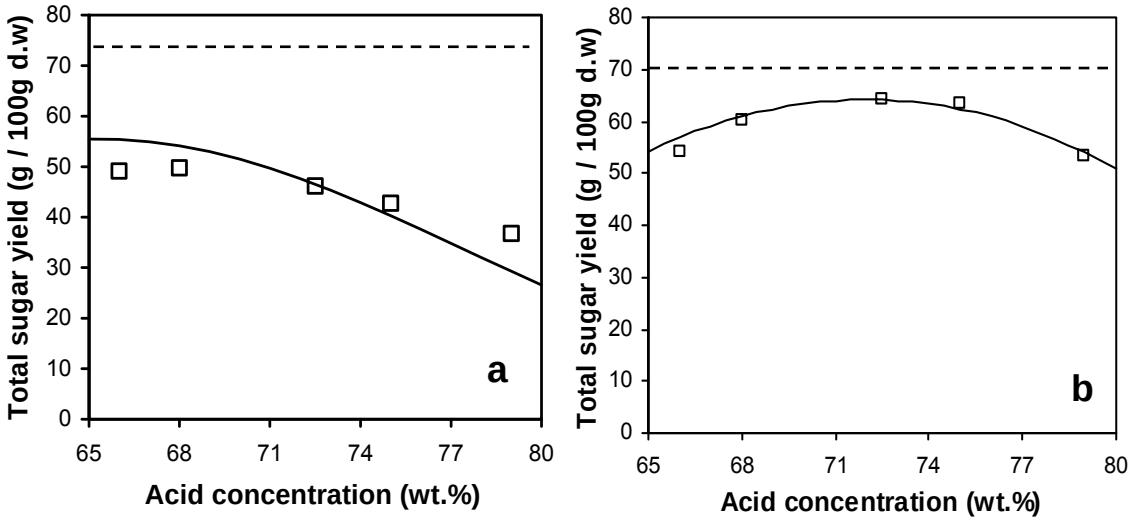

Fig. 4. Validation of the total sugar yields dependency on acid concentration at 35°C and 60 minutes. Experimental (□) versus predicted (—), Theoretical maximum (----) (a) aspen (b) pine

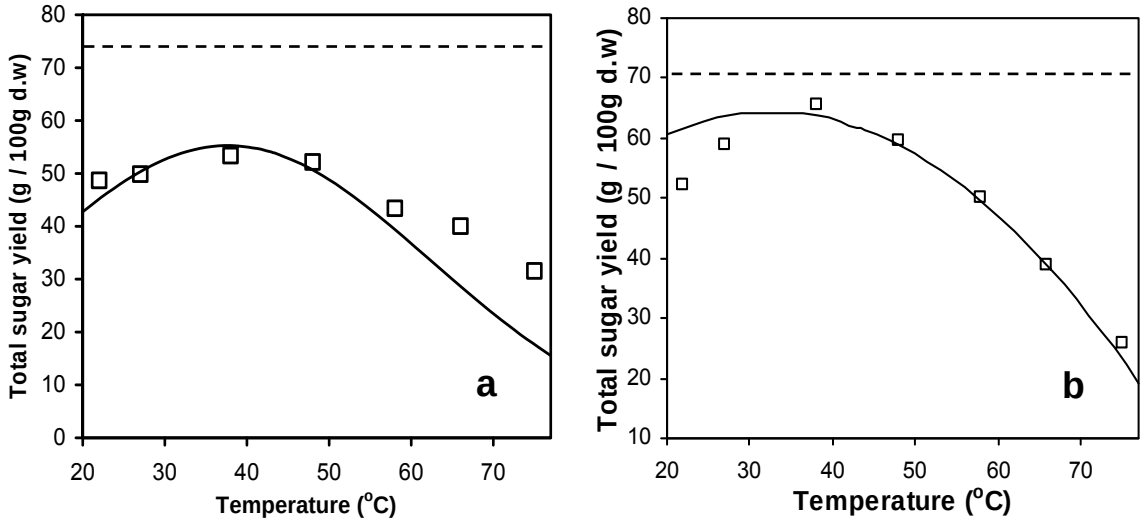

Fig. 5. Validation of total sugar yields dependency on temperature. Experimental (□) vs predicted (—),Theoretical maximum (----) (a) aspen, 67 wt.%, 60 minutes (b) pine, 72 wt.%, 60 minutes

The validity of the models is also supported by the fairly good coefficients of determination, R^2 (Table 7). This implies that the developed models in Table 7 can be reliably used for prediction and simulation of the dependency of sugar yields on process variables at various conditions within the investigated range. The decrease in conversion efficiency at higher temperatures (> 40°C) (Fig. 5) is comparable to the results reported by Clausen and Gaddy (1993), who also observed a decreased conversion to monosugars at higher temperatures and longer times in a single stage process using 70 % H_2SO_4.

Table 9 shows the predicted optimum operating window of conditions for decrystallization of both aspen and pine. The predicted maximums were obtained by running the optimizer (included in MODDE®) with the desirability function chosen to maximize the individual sugars as a desired products and minimize the sugar degradation products as the undesirable products. The prediction shows that required conditions for optimal production of individual sugars and total sugars does not necessarily minimize the formation of degradation products, suggesting a trade-off between the two when choosing the decrystallization conditions. Inspection of Table 9 interestingly shows that the maximum predicted reaction temperatures for optimum sugar yields were less than 40 °C. This is a good indication of the low-temperature requirement for concentrated acid processes and conforms to the optimum temperature of ≤ 50 °C required for enzymatic hydrolysis of cellulose to form glucose with virtually no degradation reaction (Wyman et al. 2004).

Table 9. Predicted Conditions for Optimum Yield of Sugars and Degradation Products from Sugars

	Decystallization conditions	Yield g /100 g d.w						
		Glucose	Xylose	Mannose	Galactose	Total sugar	HMF	Furfural
Aspen	39.6°C; 60min; 66.4wt.%	38.80	14.38	1.77	NS	55.6	0.17	2.64
Pine	38°C; 60min; 70 wt.%	41.02	5.68	11.10	2.16	61.14	0.24	0.62

The slight differences in response to reaction conditions predicted in Table 9 between softwoods (pine) and hardwood (aspen) can be attributed to the characteristic structural as well as chemical composition differences between the two wood species, as discussed earlier.

CONCLUSIONS

1. The developed empirical models simulated the existence of a strong correlation between major decrystallization operating variables (temperature, acid concentration, and time) and sugar yields and their subsequent degradation products. The results also showed that the reaction temperature and acid concentration were the most influential variables compared to time for both aspen and pine decrystallization within the investigated ranges. The temperature-acid concentration interaction was the most

important in both aspen and pine, implying that the two variables have more impact in the decrystallization of both aspen and pine.

2. The models can be used to predict yields of sugars and the subsequent degradation products as a function of decrystallization conditions and assess the individual effects as well as synergies of major operating variables on carbohydrate yield within the investigated ranges.
3. Although high sugar yields were achieved at optimal conditions in both aspen and pine, the formation of HMF, furfural and other undesirable products was noticeable, implying that a trade-off between minimizing the formation of degradation products and maximizing sugar yield is important in selecting decrystallization conditions.

ACKNOWLEDGMENTS

The authors would like to thank the International Office at NTNU and the NUFU project ESEPRIT for funding of this PhD study. Likewise the Nordic Energy Research (grant no. 06-renew-I34; New, innovative pretreatment of Nordic wood for cost-effective fuel-ethanol production) is thanked for giving the opportunity for collaboration on an interesting project.

REFERENCES CITED

Bayat-Makooi, F., and Goldstein, I. S. (1985). "Hydrolysis of cellulose with hydrochloric acid enhanced by cations," In: Kennedy, J. F., Phillips, G. O., Wedlock, D. J., and Williams, P. A. (eds). *Cellulose and its Derivatives: Chemistry, Biochemistry and Applications*, Ellis Horwood, Ltd, Chichester, pp. 135-141.

Bayat-Makooi, F., Singh, T. M., and Goldstein, I. S. (1985). "Some factors influencing the rate of cellulose hydrolysis by concentrated acid," *Biotechnol. Bioeng. Symp*. 15, 27-37.

Camacho, F., Gonzalez-Tello, P., Jurado, E., and Robles, A. (1996). "Microcrystalline-cellulose hydrolysis with concentrated sulphuric acid," *J. Chem. Technol. Biotechnol*. 67, 350-356.

Galbe, M., and Zacchi, G. (2007). "Pretreatment of lignocellulosic materials for efficient bioethanol production," *Adv. Biochem. Eng./Biotechnol*. 108, 41-65.

Goldstein, I. S. (1980). "The hydrolysis of wood," *Tappi.* 63(9), 141-143.

Goldstein, I. S., Pereira, H., Pittman, J. L., Strouse, B. A., and Scaringelli, F. P. (1983). "The hydrolysis of cellulose with superconcentrated hydrochloric acid," *Biotechnol. Bioeng. Symp.* 13, 17-25.

Hames, B., Ruiz, R., Scarlata, C., Sluiter, A., Sluiter, J., and Templeton, D. (2008). "Preparation of samples for compositional analysis," National Renewable Energy Laboratory, Golden, CO. USA.

Harris, E. E. (1949). "Wood saccharification," *Adv. Carbohydr. Chem*. 4, 153-188.

Heinonen, J., and Sainio, T., (2010). "Chromatographic recovery of monosaccharides for the production of bioethanol from wood," *Ind. Eng. Chem. Res*. 49 (6), 2907-2915.

Hester, R. D., and Farina, G. E. (2000). "Concentrated sulfuric acid hydrolysis of lignocellulosics," U.S. Patent No. 6063204.

Himmel, M. E., Ding, S.-Y., Johnson, D. K., Adney, W. S., Nimlos, M. R., Brady, J. W., and Foust, T. D. (2007). "Biomass recalcitrance: Engineering plants and enzymes for biofuels production," *Science* 315, 804-807.

Hon, D. N. S., and Shirashi, N. (1991). *Wood and Cellulosic Chemistry*, Marcel Dekker, New York.

Jönsson, L. J., Palmqvist, E., Nilvebrant, N. O., and Hahn-Hägerdal, B. (1998). "Detoxification of wood hydrolysates with laccase and peroxidase from the white-rot fungus *Trametes versicolor*," *Appl. Microbiol. Biotechnol*. 49, 691-697.

Knauf, M., and Moniruzzaman, M. (2004). "Lignocellulosic biomass processing: A perspective," *Int. Sugar J*. 106, 147-150.

Kunihisa, K. S., and Ogawa, H. (1985). "Acid hydrolysis of cellulose in a differential scanning calorimeter," *J. Therm. Anal.* 30, 49-59.

Liao, W., Liu, Y., Liu, C., Wen, Z., and Chen, S. (2006). "Acid hydrolysis of fibers from dairy manure," *Bioresour. Technol*. 97, 1687-1695.

Martinez, A., Rodriguez, M. E., Wells, M. L.,York, S. W., Preston, J. F., and Ingram, L. O. (2001). "Detoxification of dilute acid hydrolysates of lignocellulose with lime," *Biotechnol Prog.* 17,287-293.

McKibbins, S. W., Harris, J. F., Saeman, J. F., and Neill, W. K. (1962). "Kinetics of the acid-catalyzed conversion of glucose to 5-hydroxymethyl-2-furaldehyde and levulinic acid," *Forest Prod. J.* 12(1), 17-23.

Miller, S., and Hester, R. (2007a). "Concentrated acid conversion of pine sawdust to sugars. Part I: Use of a twin-screw reactor for hydrolysis pretreatment," *Chem. Eng. Commun.* 194, 85-103.

Miller, S., and Hester, R. (2007b). "Concentrated acid conversion of pine sawdust to sugars. Part II: High-temperature batch reactor kinetics of pretreated pine sawdust," *Chem. Eng. Commun*. 194, 103-116.

Nanguneri, D. R., and Hester, R. D. (1990). "Acid/sugar separation using ion exclusion resins: a process analysis and design," *Sep. Sci. Technol*. 25, 1829-1842.

Neuman, R. P., Rudge, S. R., and Ladisch, M. R. (1987). "Sulfuric acid-sugar separation by ion exclusion," *React. Polym*. 5, 55-61.

Neureiter, M., Danner, H., Thomasser, C., Saidi, B., and Braun, R. (2002). "Dilute acid hydrolysis of sugar cane bagasse at varying conditions," *Appl. Biochem. Biotechnol*. 98, 49-58.

Newman, R. H. (1994). "Crystalline forms of cellulose in softwoods and hardwoods," *J. Wood. Chem. Technol*. 14(3), 451-466.

Niemzl, P., Hofmann, T., and Rétfalvi, T. (2010). "Investigation of chemical changes in the structure of thermally modified wood," *Maderas Ciencia y Tecnología* 12(2), 69-78.

Nilvebrant, N. O., Reimann, A., Larsson, S., and Jönsson, L. J. (2001). "Detoxification of lignocellulose hydrolysates with ion exchange resins," *Appl. Biochem. Biotechnol.* 91-93, 35-49.

Overend, R. P., and Chornet, E. (1987). "Fractionation of lignocellulosics by steam-

aqueous pretreatments," *Philos. Trans. R. Soc. Lond. (A)* 321, 523-536.
Phaiboonsilpa, N., Lu, X., Yamauchi, K., and Saka, S. (2009). "Chemical conversion of lignocellulosics as treated by two-step semi-flow hot-compressed water," In: *Proceedings of the World Renewable Energy Congress 2009–Asia*, 235-240.
Ranjan, R., Thust, S., Gounaris, C. E., Woo, M., Floudas, C. A., von Keitz, M., Valentas, K. J., Wei, J., and Tsapatsis, M. (2009). "Adsorption of fermentation inhibitors from lignocellulosic biomass hydrolyzates for improved ethanol yield and value-added product recovery," *Micropor. Mesopor. Mat*. 122, 143-148.
Sakai, Y. (1965). "Combination of sulfuric acid with cellulose during the hydrolysis with a small amount of concentrated sulfuric acid," *Bull. Chem. Soc. Jpn*. 36, 863-868.
Schell, D. J., and Duff, B. (1996). "Review of pilot plant programs for bioethanol conversion," In: *Handb. Bioethanol: Production and Utilization*, C. E. Wyman (ed.), Taylor & Francis, Washington, D.C., 381-394.
Sheehan, J., and Himmel, M. (1999). "Enzymes, energy, and the environment: A strategic perspective on the U.S. department of energy's research and development activities for bioethanol," *Biotechnol. Prog.* 15, 817-827.
Sherrard, E. C., and Harris, E. E. (1932). "Factors influencing properties of isolated wood lignin," *Ind. Eng. Chem.* 24, 1, 103-106.
Sherrard, E. C., and Kressman, F. W. (1945). "Review of processes in the United States prior to World War II," *Ind. Eng. J.* 37, 5-8.
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., and Templeton, D. (2008a). "Determination of ash in biomass," *National Renewable Energy Laboratory*, Golden, CO, USA.
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D., (2008b). "Determination of structural carbohydrates and lignin in biomass," *National Renewable Energy Laboratory*, Golden, CO, USA.
Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., and Templeton, D. (2008c). "Determination of extractives in biomass," *National Renewable Energy Laboratory*, Golden, CO. USA.
Springfield, R. M., and Hester, R. D. (1999). "Continuous ion-exclusion chromatography system for acid/sugar separation," *Sep. Sci. Technol*. 34, 1217-1241.
Sun, Y., and Cheng, J. (2002). "Hydrolysis of lignocellulosic materials for ethanol production: A review," *Bioresour. Technol*. 83, 1-11.
Taherzadeh, M. J., and Karimi, K. (2007). "Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A review," *BioResources*. 2, 472-499.
Vaaler, D., Syverud, K., and Moe, S.T. (2001). "Characterization of pulp carbohydrates by enzymatic hydrolysis and determination of pulping yield with carbohydrate profile," *The 3rd biennial Johan Gullichsen Colloquium* 87-93.
Wen, Z. Y., and Chen, F. (2001). "Application of statistically-based experimental designs for the optimization of eicosapentaenoic acid production by the diatom *Nitzschia laevis*," *Biotechnol. Bioeng*. 75, 159-69.
Wenzl, H. F. J. (1970). "The acid hydrolysis of wood," In: *The Chemical Technology of Wood*, Academic Press Inc., New York, pp.157-252.
Weydahl, K. R. (2010). "Method of production of alcohol," *WIPO Patent Application*. WO/2010/038021

Wright, J. D., and Power, A. J. (1985). "Concentrated halogen acid hydrolysis processes for alcohol fuel production," *Biotechnol. Bioeng. Symp.* 15, 511-532.

Wyman, C. E., Decker, S. R., Himmel, M. E., Brady, J. W., Skopec, C. E., and Viikari, L. (2004). "Hydrolysis of cellulose and hemicellulose," In: S. Dumitriu (ed.), *Polysaccharides: Structural Diversity and Functional Versatility*, Second Ed., Marcel Dekker, Inc., New York, pp. 995-1033

Xiang, Q., Lee, Y. Y., Pettersson, P. O., and Torget, R. W. (2003). "Heterogeneous aspects of acid hydrolysis of α-cellulose," *Appl. Biochem. Biotechnol*. 105-108, 505-514.

Zhao, H., Kwak, J. H., Wang, Y., Franz, J. A., White, J. M., and Holladay, J. E. (2006). "Effects of crystallinity on dilute acid hydrolysis of cellulose by cellulose ball-milling study," *Energ. Fuels*. 20, 807-811.

Article submitted: September 22, 2011; Peer review completed: October 30, 2011; Revised version received and accepted: November 15, 2011; Published: November 18, 2011.