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Optimization of pretreatment conditions using full factorial design and enzymatic convertibility of shea tree sawdust

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ABSTRACT

In this study alkaline wet air oxidation (WAO), alkaline peroxide assisted wet air oxidation (APAWAO), and enzymatic hydrolysis methods were evaluated for conversion of wood residue (sawdust) to reducing sugars. Cellulose content, hemicellulose solubilization, and lignin removal for WAO pretreatment conditions were optimized by statistical analysis using a 2^3-full factorial design with reaction temperature, air pressure, and reaction time as the process parameters. An optimum WAO condition of 170 °C, 1.0 MPa, 10 min was predicted and experimentally validated to give 518 g kg^{-1} cellulose content, 580 g kg^{-1} hemicellulose solubilization, and 171 g kg^{-1} lignin removal in the solid fraction. About 7 g L^{-1} reducing sugars was detected in the pretreated liquid fraction. Presoaking the dry raw biomass for 24 h in H_2O_2 followed by wet air oxidation (APAWAO) at the optimized conditions resulted in enrichment up to 683 g kg^{-1} cellulose content in the solid fraction along with solubilization of 789 g kg^{-1} hemicellulose and 280 g kg^{-1} lignin removal. The yield of reducing sugars from WAO optimized conditions by two enzyme preparations (cellulase and β-glucosidase) was 131 mg g^{-1} of dry substrate, while the APAWAO yielded 274 mg g^{-1}. Pretreatments used in this study showed to have a disrupting effect on the lignocellulosic biomass, making the treated materials accessible for enzymatic hydrolysis. The combination of presoaking in H_2O_2 before WAO pretreatment and enzymatic hydrolysis was found to give the highest sugar yield.

1. Introduction

Bioenergy is a promising sustainable alternative for fossil fuels. It is a renewable resource and the use of biomass leads to less net CO_2 emissions over the whole life cycle compared to the fossil alternative. Because of the short life cycle, bioenergy can be considered renewable. Biomass includes all plants and plant derived materials, including agricultural crops and trees, wood and wood residues, municipal residues, and other residue materials. While most biofuels feedstock's are currently starches, oils and fats derived from the agricultural sector, whole plants and plant residues will soon be an important feedstock for lignocellulosic biofuels.

The largest potential feedstock for fuel ethanol is lignocellulosic biomass [1]. Ethanol production from such material

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can be an attractive alternative to conventional substrates [2]. Lignocelluloses mainly consist of cellulose, hemicelluloses, and lignin which are bonded together by covalent bonding, various intermolecular bridges, and van der Waals' forces forming a complex structure, making it resistant to enzymatic hydrolysis and insoluble in water [3]. Therefore, an efficient, less energy intensive and cost effective pretreatment method is a necessity for producing ethanol at economically viable cost. Pretreatment of biomass is a crucial step to overcome lignocelluloses recalcitrance in the conversion to ethanol.

Woody raw materials are generally recognized as most difficult to be hydrolysed [4] compared with other lignocelluloses. The recalcitrance of softwood to enzymatic hydrolysis is often attributed to the high lignin content and small pore size [5] and [6]. Lignin also affects enzymatic hydrolysis by irreversibly adsorbing the cellulose enzymes [7]. The simplification of lignin chemistry increases the difficulty of delignification, due to the enhanced stability of the lignin in condensed form when exposed to pretreatment conditions [8]. Woody species as softwoods such as douglas fir, red pine, specifically have lower content of pentose fractions. They have more hexoses backbone instead of xylan than the non-woody biomass and this favours their easy conversion to ethanol because fermentation of pentoses to ethanol is relatively difficult [9–12]. The woody biomass materials possess more cellulose fraction than the non-woody materials which means if pretreatment method is effective more sugar monomers will be readily available for ethanol conversion. Softwoods during enzymatic hydrolysis produce monomeric hexoses which can be fermented to ethanol easily while the fermentation of pentoses from non-woody materials is only done by a few strains [13] and [14].

Processes for bioconversion of lignocellulosic materials have been studied extensively. Examples include comminution, irradiation, steam explosion, hydrothermolysis, dilute acid, alkali, solvents, ammonia, SO_2, CO_2, and other chemicals [15].

Wet Air Oxidation (WAO) is the process of treating material with water and air or oxygen at temperatures above 120 °C and at elevated pressure [16]. One reported advantage of the wet oxidation process is the lower production of furfural and 5-hydroxymethylfurfural, which are potential inhibitors in the fermentation step [17]. In addition, wet air oxidation of biomass is a rapid reaction and takes place at lower temperatures (150 °C–200 °C) than steam explosion.

Alkaline wet oxidation pretreatment has been studied extensively for mostly agricultural residues such as; sugar cane bagasse, rice hulls, wheat straw, corn stover, rye grass, reed [18–24]. Also studies exist for wet air oxidation pretreatments for wood related sources such as yard wood wastes, softwood [25,26].

The aim of this study was to optimize the effect of different alkaline wet air oxidation conditions (reaction temperatures, reaction time and air pressures) on pretreatment of wood residue (Shea tree sawdust), and to evaluate enzymatic convertibility of the pretreated solids at the optimized conditions. Also examined was the difference between enzymatic convertibility of WAO and alkaline peroxide assisted wet air oxidation (APAWAO) treated solids.

2. Material and methods

2.1. Raw material

The Shea tree, *Vitellaria paradoxa* (classification: Angiosperm), was harvested from the forest around Idanre (6°51′N 5°06′E), south west, Nigeria in early April 2010. The tree which included the bark was pruned in the forest and chopped into size (3.66 m in length, breadth of 0.30 m, and 0.05 m in thickness) at the central processing unit of the local sawmill (Ilepa, Ifo, Nigeria; 6°49′N 3°12′E). No reliable information about the wood age can be provided since they were harvested initially for commercial purposes and the sawdust used in this study was a by-product of the processing of the wood at the sawmill. Sample storage condition before delivery to the laboratory was 27 °C–33 °C, sample condition during delivery to laboratory was air dried and thinned. The sawdust was sampled in late June, 2010 from the central processing point of the mill.

Upon arrival in the laboratory, the samples were air dried and sieved to pass through mesh 14 and retained by mesh 80 sieve sizes (BSS specification). Seventy three percent by weight of the initial sawdust was retained after the sieving process. Samples were dried in a convectional oven at 105 °C for 3 h to a dry matter content of 880 g kg^{-1} of raw biomass. The dried and sieved materials were stored in plastic bottles capped tightly and kept at room temperature. The materials were used shortly after.

2.2. Wet air oxidation pretreatment

The pretreatment was carried out in a 1.8 L volume wet air oxidation reactor (Model-4578, Floor stand HP/HT, Parr Instruments, IL., USA). 30 g of the dry biomass was mixed with 500 mL of water and 1 g $Ca(OH)_2$ (lime) was added. Air pressures at 0.5 MPa and 1.0 MPa were applied before the heating up of the suspension. The temperature was kept within ±2 °C of the set point values with constant stirring at 21 rad s^{-1}. After validating WAO optimized conditions, effects of 5 g lime loading at 140 °C and 170 °C were also investigated. For APAWAO pretreatments, 13.7 g lime loading was used to adjust the pH to 11.5 (H_2O_2 content was 34 mL L^{-1} of water). After the specified reaction time, the reactor and slurry were allowed to cool to ambient temperature. The pretreated slurry was separated into the solid and liquid fractions by vacuum filtration, and the solid fraction was washed with water to a neutral pH. The pH of the liquid fraction was measured and the solid fraction was dried and weighed. The composition of each fraction was analysed.

2.3. Analysis of the solid fraction

The compositional analysis of the raw and pretreated biomass (sawdust) is shown in Table 2.

2.3.1. Extractives

Extractives were determined by means of the Soxhlet extractor. Acetone (300 mL) was used as the solvent for extractives (5 g of dry biomass) with residence times for the

Table 1 – Statistical 2^3-factorial design for WAO experiments.

Factor	Low level	High level
Reaction temp., X_1(°C)	170	195
Air pressure, X_2 (MPa)	0.5	1.0
Reaction time, X_3(min)	10	20

boiling and rising stages equal to 70 °C and 25 min respectively for a 4 h run period. The sample was air dried for few minutes at room temperature. It was then dried at 105 °C in a convectional oven until a constant weight was obtained. The extractives content was expressed as g kg^{-1} weight of dry biomass [27–29].

Mineral components were determined by ashing at 575 °C for 6 h.

2.3.2. *Hemicellulose*

1 g of dried biomass from the extractive analysis was transferred into a 250 mL Erlenmeyer flask and then 150 mL NaOH solution (500 mol m^{-3}) was added. The mixture was boiled for 3.5 h with distilled water so as to increase the heating effect and minimize lime scales that can come from tap water. It was filtered after cooling through vacuum filtration and washed (until pH value of solution approached 7). The residue was dried to a constant weight at 105 °C. The residue was then cooled in a desiccator and weighed. The difference between the sample weight before and after this treatment is the hemicellulose content (g kg^{-1}) of dry biomass [28,29].

2.3.3. *Lignin*

300 mg of dry biomass was weighed in glass test tubes and 3 mL of 72% H_2SO_4 was added. Acid hydrolysis was allowed to occur by keeping the samples at room temperature for 2 h with mixing of samples every 30 min. 84 mL of distilled water was added to each test tube after the 2 h acid hydrolysis step bringing the total volume to 87 mL. The samples were autoclaved for 1 h at 121 °C. After the second weak acid hydrolysis step, the hydrolysates were cooled to room temperature and filtered through vaccum using a filtering crucible. The acid insoluble lignin was determined by drying the residues at 105 °C and accounting for ash by incinerating the hydrolysed samples at 575 °C in a muffle furnace. The acid soluble lignin fraction was determined by measuring the absorbance of the acid hydrolysed samples at 320 nm [30]. The lignin content was calculated as the summation of acid insoluble lignin and acid soluble lignin.

2.3.4. *Cellulose*

The cellulose content (g kg^{-1}) was calculated by difference, assuming that extractives, hemicellulose, lignin, ash, and cellulose are the only components of the entire biomass [27–29].

2.4. *Analysis of the liquid fraction*

The content of sugars in the liquid fraction was measured after 4% weak acid hydrolysis and autoclaving at 121 °C for 1 h. The samples were filtered and neutralized with calcium carbonate after the weak acid hydrolysis. Reducing sugars in the liquid fraction were estimated with DNS assay [31] using glucose as standard.

2.5. *Enzymatic hydrolysis*

The pretreated solid fractions were hydrolysed by enzymes to determine the efficiency of substrate conversion for both WAO and APAWAO. The initial dry substrate: liquid ratio was maintained at 20 g L^{-1}. Sodium citrate buffer (5 mL, 100 mol m^{-3}, pH 4.8), 0.04 mL tetracycline (10 g L^{-1} in 70% ethanol) were added to the wet pretreated substrate in 30 mL culture tubes. A commercial preparation of *Trichoderma reesei* cellulases (activity of 57.8 FPU mL^{-1}) kindly provided by M/s Zytex, Mumbai, India and β-glucosidase (activity of 10 IU mg^{-1} solid) were added at a loading of 25 FPU g^{-1} and 12.5 IU g^{-1} of dry biomass respectively. An appropriate volume of distilled water was added to bring the total volume to 10 mL. The enzymatic digestibility of pretreated solids was measured by removing 0.5 mL aliquot at 72 h experimental period. Experiments were conducted at 50 °C in a shaking incubator at 130 r min^{-1} [32]. To quench the hydrolysis, the samples were boiled for 15 min in a water bath

Table 2 – Composition of solid in g kg^{-1} and liquid fractions of WAO treated sawdust.

Reaction products	Raw dried sawdust	(I) 170 °C 0.5 MPa 10 min	(II) 170 °C 0.5 MPa 20 min	(III) 170 °C 1.0 MPa 10 min	(IV) 170 °C 1.0 MPa 20 min	(V) 195 °C 0.5 MPa 10 min	(VI) 195 °C 0.5 MPa 20 min	(VII) 195 °C 1.0 MPa 10 min	(VIII) 195 °C 1.0 MPa 20 min
Solid fraction									
Dry matter		921	910	824	787	798	747	737	747
Extractives	19	25	28	36	56	52	34	65	61
Cellulose	459	565(1134)	569(1128)	563(1012)	491(842)	515(897)	518(845)	459(738)	428(696)
Hemicellulose	203	81(366)	99(444)	106(429)	133(515)	91(357)	112(411)	139(503)	171(629)
Lignin	299	315(970)	293(890)	276(759)	307(808)	331(884)	320(799)	313(770)	324(809)
Ash	20	14	12	20	14	11	16	25	16
Liquid fraction									
RS (g L^{-1})	–	6.6	10.2	6.1	13.5	18.2	17.2	24.5	19.0
pH	12.7	7.6	7.6	7.7	6.9	6.5	6.7	6.7	6.2

and then cooled in an ice bath. After hydrolysis the samples were centrifuged at 2254 gravities for 5 min to remove residual solids. Fermentable sugars were estimated as reducing sugars with DNS assay [31]. The amount of total reducing sugars was calculated [33] as follows:

Reducing sugar yield from enzymatic hydrolysis (mg g^{-1} of dry biomass) = (amount of reducing sugar produced after hydrolysis)/(amount of treated dry biomass).

2.6. Statistical design and optimization

A statistical 2^3-factorial design was used for the design of experiment and the process optimization of wet air oxidation pretreatment of sawdust using lime (Table 1). Three operating factors viz. reaction temperature, air pressure and reaction time were taken into consideration, to yield 8 different experiments [34]. The choice of these three factors was based on earlier reported studies on rice husks and wood waste [19,26]. The three variables chosen were designated as X_1 (temperature), X_2 (pressure), X_3 (reaction time).

The model generated as a function of these variables on the predicted responses of cellulose content, hemicelluloses solubilization, and lignin removal is a second-order polynomial and is represented as follows:

$$Y = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_{1,2} X_1 X_2 + \alpha_{1,3} X_1 X_3 + \alpha_{2,3} X_2 X_3 \quad (1)$$

The predicted responses are designated as Y associated with each factor level combinations; α_0 to $\alpha_{2,3}$ are the regression coefficients; X_1,X_2,X_3 are the factors. The optimal process parameters for WAO were estimated by MINITAB 15 software (PA, USA). The coefficients in the second-order polynomial (Eq. (1)) were calculated by multiple regression analysis, based on the experimentally obtained data, and then the predicted responses were obtained using Eq. (1). The equations were validated using analysis of variance (ANOVA) method. Response surfaces were drawn to determine the individual and interactive effects of the test variables on cellulose content, hemicelluloses solubilization, and lignin removal. The optimal values of each factor to optimize the process responses were based on Multi-Objective Numerical Optimization. The order in which the experiments were carried out was randomized. All the experimental runs were performed within one week. Each experiment was replicated twice; reported results indicate the average values of the replicated experiments.

3. Results and discussion

3.1. Composition of the solid fraction

The WAO pretreatment was aimed at fractionating the wood biomass residue (sawdust) into a solid fraction containing as much cellulose and as less lignin as possible; and a liquid fraction containing solubilized hemicellulose the best preserved as possible [18]. This work was therefore used to study the enzymatic convertibility (at optimized pretreatment conditions) of the biomass subjected to wet air oxidation

Table 3 – Experimental and predicted values for solid and liquid fractions of WAO treated sawdust.

WAO condition	Solid fraction (g kg^{-1})				Liquid fraction					
	Cellulose content		Lignin removal		Hemicellulose solubilization		Reducing sugar (g L^{-1})		pH	
	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
I	565	570	30	31	634	626	6.6	5.6	7.6	7.6
II	569	564	110	109	556	564	10.2	11.3	7.6	7.6
III	563	558	241	240	571	579	6.1	7.1	7.7	7.7
IV	491	496	192	193	485	477	13.5	12.5	6.9	6.9
V	515	510	116	115	643	651	18.2	19.3	6.5	6.5
VI	518	523	201	202	589	581	17.2	16.2	6.7	6.7
VII	459	464	230	231	497	489	24.5	23.4	6.7	6.7
VIII	428	423	191	190	371	379	19.0	20.1	6.2	6.2

pretreatment method using lime as the chemical catalyst as against the traditional use of Na_2CO_3 or H_2SO_4 [18,20,24].

It has been well recognized that woody raw materials especially softwoods are generally more recalcitrant to enzymatic hydrolysis than other lignocellulosic substrates such as agricultural residues. Similar results were also observed in this study. However, subjecting the substrate to some pretreatment variations could increase its suitability for efficient enzymatic hydrolysis. The percentage of dry biomass recovered in the solid fraction after WAO pretreatment ranged from 737 g kg^{-1}–921 g kg^{-1} of dry substrate and it was higher for pretreatments at 170 °C than for pretreatments at 195 °C (Table 2).

Higher temperature (195 °C), when combined with high pressure and/or reaction time were found to result in excessive biomass charring which was apparent from the black colour of the solid material in pretreatments (VI), (VII) and (VIII) (Table 2). The biomass pretreated at 170 °C ((I)–(IV)) had a brown colour and a fluffy consistence. In the pretreatment conditions (I)–(VI) cellulose recovery of the solid fraction increased (recovery of the components in the solid fraction is shown in parentheses in Table 2). At severe conditions (VII) and (VIII), cellulose recovery decreased indicating undesirable cellulose degradation. The thermal degradation of cellulose is said to begin on increasing the temperature from around 195 °C–200 °C [35]. Hemicellulose recovery varied with all the conditions. High values of hemicellulose recovery occurred at high temperature pretreatments (VII) and (VIII). Increased cellulose content in the pretreated biomass ranged from 515 g kg^{-1}–565 g kg^{-1} compared with the initial raw biomass of 459 g kg^{-1}. Cellulose content decreased at elevated temperature and high air pressure to 428 g kg^{-1} due to degradation of cellulose. Cellulose enrichment was due majorly to hemicellulose solubilization and a small percentage of lignin removal. Hemicellulose solubilization varied from 371 g kg^{-1}–643 g kg^{-1} weight of dry biomass. The lignin removal was very low in all the conditions with the highest value of 241 g kg^{-1} (pretreatment (III)). This can be attributed to the high lignin content in the raw biomass (i.e. a lignin content of 299 g kg^{-1}) [5] and [25]. Under these experimental conditions, it was revealed that more hemicellulose is solubilized than lignin removal.

The second-order polynomials obtained were as follows:

$$\text{Cellulose content}\,(\text{g kg}^{-1}) = 857.25 - 1.9X_1 + 533.80X_2 - 8.75X_3 - 2.64X_1X_2 + 0.08X_1X_3 - 11.00X_2X_3$$

$$R^2 = 0.9884 \quad (2)$$

$$\text{Hemicellulose solubilization}\,(\text{g kg}^{-1}) = -311.40 + 5.92X_1 + 1550X_2 + 3.24X_3 - 9.20X_1X_2 - 0.032X_1X_3 - 8.00X_2X_3$$

$$R^2 = 0.9909 \quad (3)$$

$$\text{Lignin removal}\,(\text{g kg}^{-1}) = -1552.28 + 6.87X_1 + 1957.70X_2 + 15.43X_3 - 7.57X_1X_2 + 0.03X_1X_3 - 25.30\,X_2X_3$$

$$R^2 = 0.9999 \quad (4)$$

When the values from X_1 to X_3 were substituted in the above equations, the predicted responses were obtained (Table 3).

3.2. Statistical analysis of results

The statistical treatment combinations of the test variables along with the measured response values, corresponding to all combinations are summarized in Table 4.

The *P*-values (probability values) are used as tools to check the significance of each of the coefficients in the models, which in turn, may indicate the patterns of the interaction among the variables. The larger the magnitude of *T* and smaller the *P*-value the more significant is the corresponding coefficient. From Table 4, temperature is marginally significant on cellulose content ($P = 0.099$), pressure also is marginally significant on hemicellulose solubilization ($P = 0.081$). All the main effects and the interaction effects are significant on lignin removal ($P < 0.05$); except for temperature and time interaction. The summary of analysis of variance (ANOVA) representing the results is discussed in Table 5.

ANOVA is required to test the significance and adequacy of the models. The Fisher's variance ratio (*F*-value) is the measure of variation in the data about the mean. Here the ANOVA of the multiple regression revealed that the quadratic models derived from the factorial design could adequately be used to predict the responses as evident from the high *F*-values. In addition, the multiple correlation coefficients (R^2) of the regression equations obtained from ANOVA were 0.9884 for cellulose content, 0.9909 for hemicellulose solubilization, and 0.9999 for lignin removal. This means that the models fitted well with the experimental data. The R^2-value for cellulose content implies that the sample variation of 98.8% is attributed to the factors, and also indicates that only 1.2% of the total variation is not explained by the model. For hemicellulose solubilization (R^2 = 99.1%), only 0.9% of the total variation is not explained by the model. R^2-value for lignin

Table 4 – Estimated *t*-values and *p*-values for the regression coefficients.

	t-value			*p*-value		
	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose	Lignin
Constant	97.81	67.91	262.20	0.007	0.009	0.002
X_1	−6.38	−2.28	33.00	0.099	0.263	0.019
X_2	−5.38	−7.78	79.40	0.117	0.081	0.008
X_3	−2.29	−5.38	15.40	0.263	0.117	0.041
X_1X_2	−1.57	−3.59	−37.80	0.361	0.173	0.017
X_1X_3	0.95	−0.25	3.00	0.516	0.844	0.205
X_2X_3	−2.62	−1.25	−50.60	0.232	0.430	0.013

Table 5 – Analysis of variance (ANOVA) for polynomial model obtained from experimental design.

Cellulose content					
Source	DF	SS	MS	F	P
Main effect	3	16514.5	5504.8	24.97	0.146
2 - way interaction	3	2257.0	752.3	3.41	0.374
Residual error	1	220.5	220.5		
Total	7	18992.0			
Hemicellulose Solubilization					
Main effect	3	48457.0	16152.3	31.55	0.130
2 - way interaction	3	7444.5	2481.5	4.85	0.319
Residual error	1	512.0	512.0		
Total	7	56413.5			
Lignin Removal					
Main effect	3	238.45.4	7948.46	2543.51	0.015
2 - way interaction	3	12494.4	4164.79	1332.73	0.020
Residual error	1	3.1	3.12		
Total	7	36342.9			

DF = Degree of Freedom. SS = Sum of squares. MS = Mean Square.

removal (99.9%) reveals that 0.1% of the total variation is not explained by the model. The predicted values were compared with the experimentally obtained values and the data were in close agreement (Table 3).

Two-dimensional contour plots and three-dimensional response surface curves were plotted to study the interactions between the various parameters in WAO pretreatment of the sawdust material and were used to determine the optimum levels of each factor required to obtain maximum responses. Effects of individual factors on cellulose content, lignin removal, and hemicellulose solubilization of the solid fraction are shown in Fig. 1.

Fig. 1(A–C) shows the contour and surface plots of the interaction effect of air pressure, reaction time, and temperature on cellulose content, hemicellulose solubilization, and lignin removal to obtaining maximum responses. Fig. 1(A1 and A2) shows the effects of temperature, air pressure, and time on cellulose content. The air pressure should be kept at high value (1.0 MPa), the reaction time maintained at low value (10 min), and the reaction temperature also kept at a low value (170 °C) to obtain the maximum value of cellulose content in the solid fraction. Fig. 1(B and C) shows the surface plots of effect of air pressure and reaction time on hemicellulose solubilization and lignin removal when the reaction temperature is kept at low level.

3.3. *Composition of the liquid fraction*

Sugars in the liquid fraction as reducing sugars (RS) increased at high temperature pretreatments. This was as a result of cellulose degradation/solubilization from the solid fraction at the

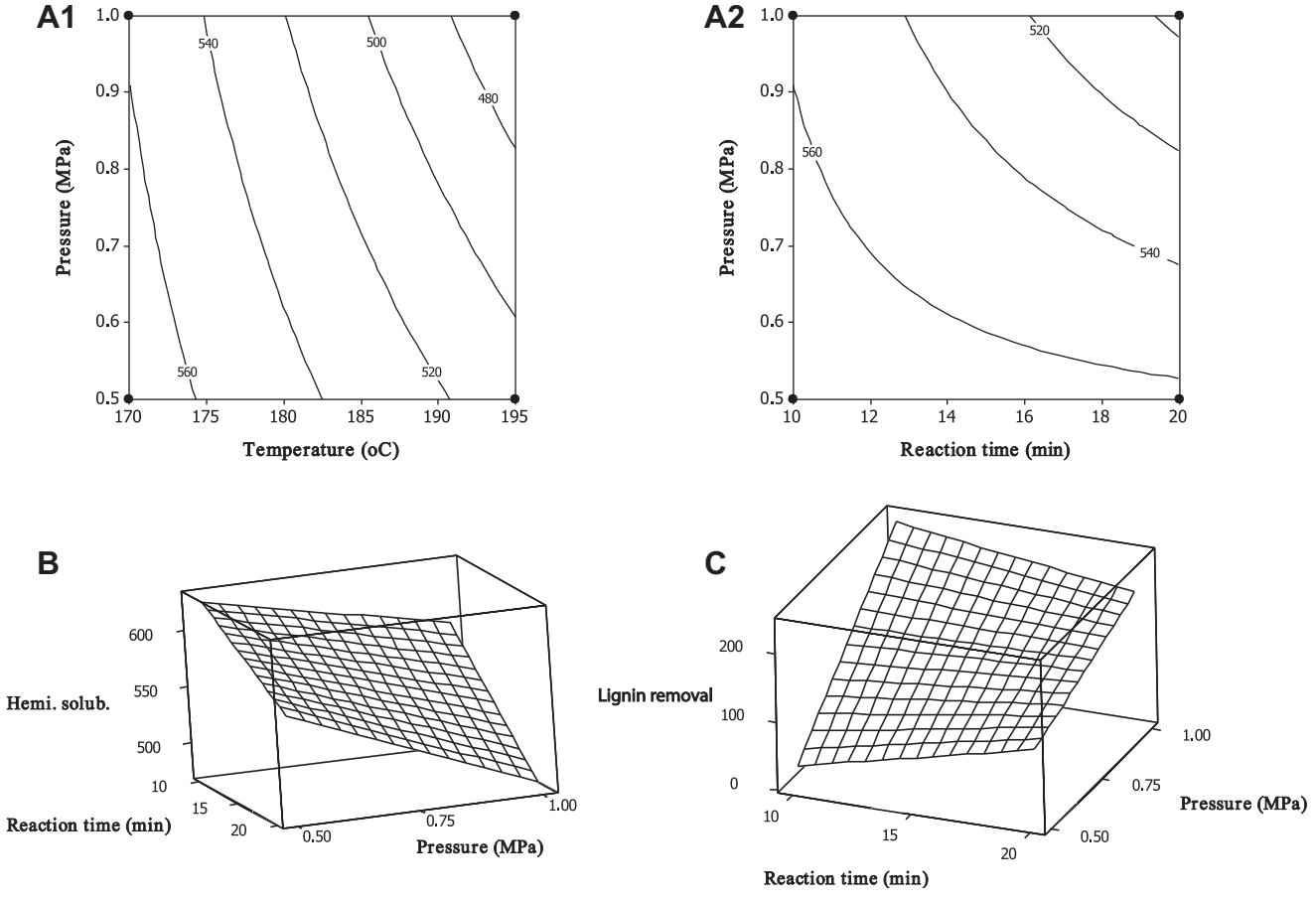
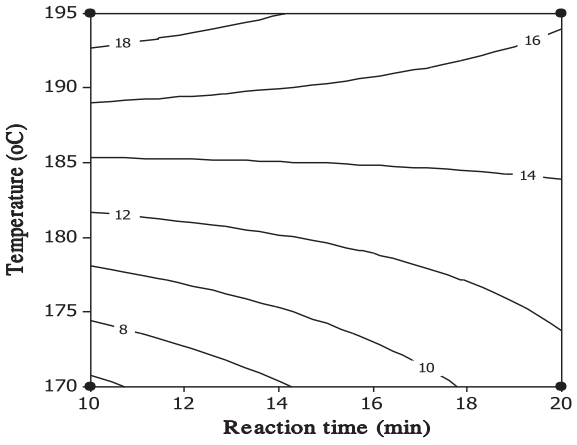
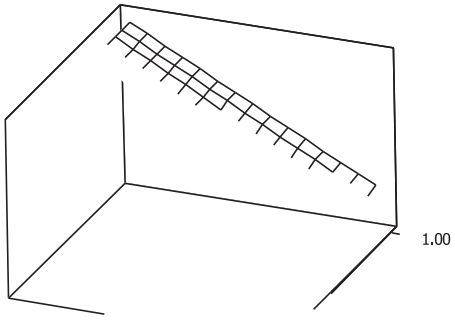


Fig. 1 – Contour and surface plots of the responses in the solid fraction of the pretreated sawdust. (A1) contour plot of cellulose content (g kg^{-1}) vs. Pressure and temperature; (A2) contour plot of cellulose content (g kg^{-1}) vs. Pressure and time; (B) Surface plot of hemicellulose solubilization (g kg^{-1}) vs. Reaction time and pressure; (C) Surface plot of lignin removal (g kg^{-1}) vs. Reaction time and pressure.





high temperature (195 °C). Pretreatment (VII) gave the highest reducing sugars value of 25 g L^{-1} while low temperature (pretreatment (III)) gave the lowest value of 6 g L^{-1}. The pH of the liquid fraction of the pretreated biomass showed a progressively decreasing trend with increasing temperature, pressure, and reaction time. The decreasing trend was reflected at high temperature range with the lowest pH of 6.2 (pretreatment VIII).

Multiple regression analysis was performed on the experimentally obtained data for the concentration of reducing sugar, and pH of the liquid fraction. The regression coefficients.

Obtained were used to build the model equations to predict the response Y as explained in Eq. (1). The second-order polynomials obtained were:

$$\text{Reducing sugars}\,(\text{g L}^{-1}) = 14.429 + 5.311X_1 + 1.353X_2 + 0.576X_3 + 0.651X_1X_2 - 2.179X_1X_3 - 0.075X_2X_3$$

$$(R^2 = 0.971) \quad (5)$$

$$\text{pH} = 6.98 - 0.458X_1 - 0.108X_2 - 0.138X_3 + 0.065X_1X_2 + 0.070X_1X_3 - 0.175X_2X_3$$

$$(R^2 = 1.000) \quad (6)$$

The closeness of the predicted and experimental values of reducing sugars and pH (Table 3) is consistent with the high R^2 values in Eqs. (5) and (6).

Contour and surface plots of the responses were plotted to investigate the main and interaction effects of the process variables and to optimize the WAO process (Fig. 2(A and B)). Reducing sugar concentration was found to be higher at high temperature and short time conditions (VII). However lower concentrations of reducing sugars (which has to correspond to increase in cellulose content, and a higher lignin removal in the solid fraction) will be preferable to higher concentration, and a low pH value.

3.4. *Optimization of the pretreatment conditions*

The model equations for the various responses (Eqs. (1)–(6)), and the response surface and contour plots were utilized in determining the optimum WAO conditions so as to obtain a solid fraction with high cellulose content, low lignin and hemicellulose, and a liquid faction with low concentrations of reducing sugars, at a reasonably mild pH. With all these constraints in mind, the optimum cumulative responses were obtained at 170 °C, 1.0 MPa, and 10 min (Table 6(A)). Additional sets of WAO experiments at these specific conditions were performed to validate the optimized conditions. The experimental and predicted responses were found to be in close agreement, thus confirming the optimization process.

In this study, the optimized conditions revealed a low lignin removal. The pretreatments examined after the WAO optimized conditions (Pretreatments B to E) (Table 6) were to find out if more lignin can be solubilized or removed from the solid fraction thereby increasing enzymatic yield of pretreated biomass. Conditions such as increasing lime loading, reaction temperature at 140 °C, H_2O_2 addition just before WAO pretreatment, and presoaking the dry biomass for 24 h in H_2O_2 followed by WAO process were considered. Under these variations, 683 g kg^{-1} cellulose content, 789 g kg^{-1} hemicellulose solubilization, and 280 g kg^{-1} lignin removal of the dry biomass was achieved in the solid fraction obtained at pretreatment E. At 140 °C and 5 g lime loading (pretreatment D), cellulose content and hemicellulose solubilization were comparable to pretreatment at 170 °C and 5 g lime loading (pretreatment B).

The enzymatic digestibility of biomass is affected by the pretreated methods used and the structural modification of the biomass (e.g. lignin content, acetyl group content, and crystallinity) [36]. Fig. 3 shows the TRS (total reducing sugars) yields (reducing sugars yield in mg g^{-1} dry biomass) after hydrolysis of pretreated sawdust at 50 °C and 72 h for pretreatments A to E (Table 6).

It can be noted that under the experimental conditions used maximum TRS yield obtained was 274 mg g^{-1} dry biomass (Pretreatment E) (170 °C, 1.0 MPa, H_2O_2 content of 34 mL L^{-1} of water, 13.7 g lime loading, and 10 min). Pretreatment A (170 °C, 1.0 MPa, 1.0 g lime loading, and 10 min), which was the validated pretreatment condition for WAO gave the lowest TRS yield of 131 mg g^{-1} dry biomass. Pretreatment at 170 °C, 1.0 MPa, 5 g lime loading, 10 min (pretreatment B) showed little increase on TRS yield (159 mg g^{-1} dry biomass) to pretreatment at 140 °C, 1.0 MPa, 5 g lime loading, 10 min (TRS yield of 147 mg g^{-1} dry biomass). The low TRS yields under these experimental conditions may be primarily due to the low lignin removal from the pretreatment step caused by the high lignin content in the raw biomass. Also, β-glucosidase is said to be inhibited by glucose [37]. Inhibition of the enzymes by the end products negatively affects cellulose hydrolysis. However, the optimal conditions change with the hydrolysis residence time, temperature [38] and are also dependent on the source of the enzymes. The concentration of cellulases also has a high impact on the conversion of the cellulose.

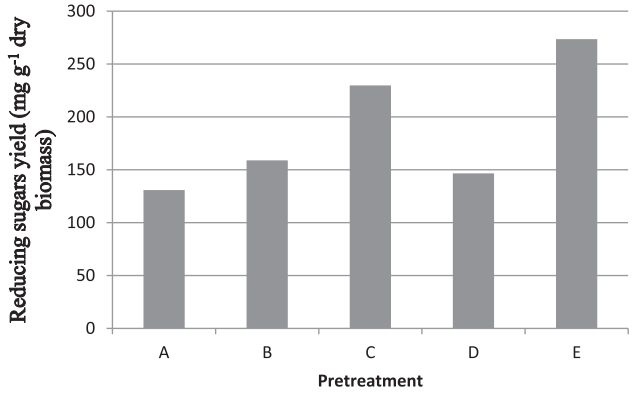


Fig. 3 – Enzymatic yield results for alkaline wet air oxidation (A, B, and D) and alkaline peroxide assisted wet air oxidation pretreated sawdust (C and E).

4. Conclusions

These investigations revealed that WAO could be used to pretreat sawdust high in lignin content to an appreciable level amenable to enzymatic hydrolysis. Validated optimized conditions of 170 °C, 1.0 MPa, 1 g lime loading and 10 min gave a result of 518 g kg^{-1} cellulose content, 580 g kg^{-1} hemicellulose solubilization, and 171 g kg^{-1} lignin removal. Enhancing the optimized conditions by soaking the raw material in alkaline peroxide (13.7 g lime and H_2O_2 content of 34 mL L^{-1} of water) for 24 h before WAO pretreatment resulted in 683 g kg^{-1} cellulose content, 789 g kg^{-1} hemicellulose solubilization, and 280 g kg^{-1} lignin removal. From the enzymatic digestibility results, increased lime loading, hydrogen peroxide addition, and maintaining the temperature at 170 °C, reaction time of 10 min, and 1.0 MPa air pressure increased the TRS yields of the WAO process on the wood residue. However, a further evaluation is needed to know the optimum level of lime, and hydrogen peroxide to be added during the pretreatment process. As a result of the recalcitrant nature of woody materials the lignin fraction of the substrate remained mainly undissolved; this could be available for energy production by combustion.

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