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Hydrogen peroxide and lime based oxidative pretreatment of wood waste to enhance enzymatic hydrolysis for a biorefinery: Process parameters optimization using response surface methodology

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HIGHLIGHTS

- Hydrogen peroxide and lime based oxidative pretreatment of wood waste.
- Response surface methodology was used to optimize the process parameters.
- Optimum conditions of 150 °C, 1% H_2O_2, and 45 min were predicted and validated.
- 59% (w/w) cellulose content was retained in the solid fraction.
- Reducing sugars yield from pretreated material was up to 263.49 mg g^{-1} dry biomass.

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ABSTRACT

Response surface methodology (RSM) was adopted for the optimization of process variables in the alkaline peroxide oxidation (APO) pretreatment of *Vitellaria paradoxa* sawdust based on central composite design (CCD) experiments. A 2^3 five level CCD with central and axial points was used to develop a statistical model for the optimization of process variables. Maximum response for the pretreatment was obtained when applying the optimum values for temperature (150 °C), time (45 min), and 1% (v/v) H_2O_2. At the optimum conditions, up to 70% of the initial hemicellulose was removed in treatments, which also caused some delignification (up to 11% of the initial lignin was removed), whereas cellulose was almost quantitatively retained in the solid phase. Alkaline peroxide assisted wet air oxidation (APA-WAO) pretreatment at the optimum conditions resulted in enrichment up to 60% cellulose content along with solubilization of 80% hemicellulose and 17% of lignin initially present in the raw sawdust. Reducing sugars yield after 72 h enzymatic hydrolysis of pretreated biomass at optimized APO conditions was 177.89 mg equivalent glucose g^{-1} dry biomass. Addition of 10 bar air pressure at the optimized pretreatment conditions increased the sugars yield to 263.49 mg equivalent glucose g^{-1} dry biomass.

1. Introduction

Ethanol production from lignocellulosic residues has potential to significantly improve sustainability of biofuels for transport by avoiding land-use competition with food crops and reducing impacts related to agricultural inputs. However, high production costs remain bottleneck for large scale development of this pathway [1]. Cellulose is recalcitrant to biodegradation and needs to be hydrolyzed in an initial pretreatment step into its constituent cellobiose units and into simpler D-glucose units in order to be liable to biochemical conversion. The rate and extent to which cellulose in lignocellulosic materials can be enzymatically saccharified is limited by two important factors: the close physical and chemical association between lignin and the cell wall polysaccharides, and the degree of crystallinity within the cellulose polymer itself [2]. In order to hydrolyze lignocellulosic biomass with enzymes successfully, it is also important to apply a suitable pretreatment that can effectively disrupt linked lignin and crystalline cellulose. Certain kinds of chemical, physical and/or biological pretreatments remove or disrupt lignin sheath, reduce the degree of cellulose crystallinity, remove or separate hemicellulose from cellulose and increase the accessible surface area of biomass, resulting in an enhancement of lignocellulosic substrate digestibility.

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Hydrogen peroxide and alkaline oxidative pretreatment (alkaline peroxide oxidation (APO)) is known to decrystallize cellulose [3]. It is also known that under proper conditions hydrogen peroxide (H_2O_2) will react readily with lignin and related phenolics [4,5] to yield an array of low molecular weight, water-soluble oxidation products [6]. Natural degradation of lignin can occur through a variety of different organisms. Hydrogen peroxide excreted by the organism plays an important role in the degradation [7,8]. The mechanism by which alkaline peroxide pretreatment enhances enzymatic saccharification appears to involve both a release of lignin from the lignocellulosic matrix and a dramatic increase in the degree of hydration of the cellulose polymer [9]. The APO process (an alternative oxidative treatment to air or oxygen delignification) has been shown to be effective in increasing the digestibility of crop residues [3]. Extensive studies exist on the APO process pretreatment of agricultural residues [9–12].

The conventional technique for the optimization of a multi-factorial system is to deal with one-variable at a time (OVAT). However, this type of method is time-consuming and also does not reveal the interactive and square effects hence; response surface methodology (RSM) which is based on statistical principles was employed as a strategy to study individual as well as interactive effects of the parameters and also to generate significant information regarding the optimum sets of levels of the pretreatment process.

In the present study attempts were made to find out whether the APO process could be used for delignification (lignin removal) and to enhance the digestibility of high lignin wood waste (sawdust). Also investigated was if lime can be used as a viable alternative source of alkali for sodium hydroxide (a conventional alkali source of the APO process), thereby reducing the cost of treatment. RSM based on central composite design (CCD) of experiments was adopted to investigate the optimum parameters of APO pretreatment such that the cellulose content, the hemicellulose solubilization, and delignification will enhance enzymatic hydrolysis. The effects of three process parameters (reaction temperature, reaction time, and percent hydrogen peroxide) on the pretreatment step were studied. The most suitable APO conditions to obtain enriched solid fraction for enzymatic digestibility were selected after optimizing and validating the pretreatment conditions.

Further variations at the optimized conditions; alkaline wet air oxidation (WAO), and alkaline peroxide assisted wet air oxidation (APAWAO) were compared with the APO process. The enzymatic hydrolysis of biomass was also investigated to ascertain the efficiencies of pretreatments.

2. Material and methods

2.1. Raw material

Vitellaria paradoxa sawdust raw biomass was carefully collected from a sawmill in Ifo town, south west Nigeria. Samples were sieved to yield fine particles. The fraction which passes through BSS 14 and retained by BSS 80 mesh sizes was used in all the experiments. The average particle sizes therefore varied between 0.09 and 0.51 mm, which made up 73% of the harvested raw material. Samples were dried in a convectional oven at 105 °C for 3 h to a dry matter content of 88%. The sieved and dried samples were stored in rubber bottles capped tightly and kept in a locker. The materials were used shortly after.

2.2. Alkaline peroxide oxidation pretreatment

All pretreatment experiments were carried out in a Parr reactor model 4578. This 1.8 L batch reactor fitted with double six-blade turbine impellers was equipped with an external heating element sheathed with a jacket and internal stainless steel loops for cooling. 30 g of dry substrate were mixed with 500 mL distilled water containing 30%(w/v) H_2O_2 (0.84%, 1.00%, 1.25%, 1.50%, 1.66%) and adjusted to pH 11.5 with respective lime loadings (9.0, 13.7, 17.5, 20.3, 30.0 g). Slurries were pretreated at different temperatures, and at different time intervals (Table 1).

Table 1
Statistical 2^3 – central composite design for APO experiments.

Factor	Low level	High level
Reaction temp., X_1 (°C)	120	150
Reaction time, X_2 (min)	20	40
H_2O_2, X_3 (%)	1	1.5

The reaction was controlled by a Parr PID controller model 4857. A solenoid valve adjusted the water flow through the internal coil, and regulated the temperature at the set point (±2 °C) with constant stirring at 21 rad s^{-1}. Each reaction was terminated by running cold water through the internal loops. There was constant stirring while cooling, thereby maintaining a relatively homogenous environment. After the specified reaction time, the reactor and slurry were allowed to cool to ambient temperature. Specific lime consumption (g $Ca(OH)_2$ g^{-1} dry biomass) for each reaction time was determined through pH titration (neutralizing slurry with 5 N HCl) [13]. The pretreated slurry was separated into the solid and liquid fractions by vacuum filtration, and the solid fraction was washed with water. The solid fraction was dried and weighed. The solid fraction was used to determine the optimum conditions for pretreatment.

2.3. Analysis of the solid fraction

The compositional analysis of the raw, pretreated, and specific lime consumption of biomass is given below (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, w_o, g) with residence times for the 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 (w_1, g) was obtained. The extractives weight percent, % (w/w) is calculated as given in Eq. (1) [14–16]. Mineral components were determined by ashing at 575 °C for 6 h.

$$\%(\mathrm{w/w}) = \{(w_o - w_1)/w_o\} \times 100\% \quad (1)$$

2.3.2. Hemicellulose

One gram of dried biomass from the extractive analysis was transferred into a 250 mL Erlenmeyer flask and then 150 mL NaOH solution (0.5 mol L^{-1}) was added. The mixture was boiled for 3.5 h with distilled water so as to increase the heating effect and minimize lime scales. 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 [15,16].

2.3.3. Lignin

Three hundred milligrams of dry biomass was weighed in glass test tubes and 3 mL of 72% H_2SO_4 was added. Acid hydrolysis was made 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

Table 2
Chemical composition of raw, pretreated, and specific lime consumption of sawdust.

	Dry matter yield	Extractives	Cellulose[a]	Hemicellulose[a]	Lignin[a]	Ash	Lime consumption[b]
Raw biomass	100	1.89	45.86	20.31	29.90	2.04	
Run order							
1	90.58	4.17	53.71 (106.08)	8.59 (38.31)	31.65 (95.88)	1.88	0.06
2	90.30	5.11	55.49 (109.26)	8.98 (39.93)	28.88 (87.22)	1.62	0.07
3	85.96	4.86	52.71 (98.80)	9.79 (41.43)	30.53 (87.77)	2.11	0.12
4	87.83	4.51	53.81 (103.06)	9.83 (42.51)	30.05 (88.27)	1.80	0.11
5	90.28	4.37	53.57 (105.46)	10.13 (45.03)	30.23 (91.28)	1.70	0.13
6	90.02	5.41	51.47 (101.03)	9.98 (44.23)	31.30 (94.23)	1.84	0.07
7	91.46	4.00	54.48 (108.65)	9.83 (42.27)	29.78 (91.09)	1.91	0.11
8	88.96	5.82	54.07 (104.88)	8.01 (35.08)	30.38 (90.39)	1.72	0.09
9	94.46	11.70	46.85 (96.50)	8.98 (41.77)	30.48 (96.29)	1.99	0.10
10	92.71	14.06	42.96 (86.85)	9.83 (44.87)	30.69 (95.16)	2.46	0.06
11	89.43	5.17	53.58 (104.48)	9.12 (40.16)	29.71 (88.86)	2.42	0.09
12	87.18	9.43	48.95 (93.05)	8.60 (36.92)	30.59 (89.19)	2.43	0.11
13	88.62	5.13	53.10 (102.61)	9.29 (40.54)	29.76 (88.21)	2.72	0.11
14	85.95	5.35	54.05 (101.30)	7.78 (32.92)	30.52 (87.73)	2.12	0.12
15	87.27	7.67	52.59 (100.08)	6.88 (29.56)	31.48 (91.88)	1.38	0.09
16	87.41	7.85	51.44 (98.05)	7.35 (31.63)	31.44 (91.91)	1.92	0.08
17	91.89	5.89	53.14 (106.48)	9.17 (41.49)	29.82 (91.64)	1.98	0.09
18	88.67	5.78	52.82 (102.13)	8.98 (39.21)	30.29 (89.83)	2.13	0.10
19	91.14	5.98	51.75 (102.85)	9.20 (41.74)	31.06 (94.68)	2.01	0.08
20	87.98	5.11	53.15 (101.97)	9.75 (42.24)	29.78 (87.63)	2.21	0.09

[a] Recovery of components are in parentheses.
[b] Lime consumption in g $Ca(OH)_2$ g^{-1} dry biomass.

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 residue at 105 °C and accounting for ash by incinerating the hydrolyzed samples at 575 °C in a muffle furnace. The acid soluble lignin fraction was determined by measuring the absorbance of the acid hydrolyzed samples at 320 nm [17].

2.3.4. Cellulose

The cellulose content was calculated by difference, assuming that extractives, hemicellulose, lignin, ash, and cellulose are the only components of the entire biomass [14–16].

$$\%W_{\text{cellulose}} = 100 - (\%W_{\text{ash}} + \%W_{\text{extractives}} + \%W_{\text{hemicellulose}} + \%W_{\text{lignin}}) \quad (2)$$

where $\%W$ is the % (w/w) of lignocellulosic components analyzed.

2.4. Enzymatic hydrolysis

The wood waste was hydrolyzed by enzymes to determine the efficiency of substrate conversion for APO, WAO, and APAWAO pretreatments. The initial dry substrate : liquid ratio was maintained at 20 g L^{-1} in 30 mL culture tubes. The pH was adjusted to 4.8 by adding 5 mL of a 100 mol m^{-3} sodium citrate buffer. 0.04 mL tetracycline (10 g L^{-1} in 70% ethanol) was added to prevent microbial growth in the mixture. Cellulase activity (*Trichoderma reesei*, kindly provided by M/s Zytex, Mumbai, India) was measured by the filter paper method as described by Ghose [18] and was 57.8 FPU mL^{-1}. β-glucosidase activity (Himedia Laboratories Pvt. Ltd., Mumbai, India) was 10 IU mg^{-1} solid. The enzyme loadings were 25 FPU g^{-1} dry biomass of cellulase and 12.5 IU g^{-1} dry biomass of β-glucosidase. An appropriate volume of distilled water was added to bring the total volume to 10 mL. The enzymatic hydrolysis of pretreated solids was measured by removing 0.5 mL aliquot at 2, 24, and 72 h experimental periods. Experiments were conducted at 45 °C in a shaking incubator at 14 rad s^{-1} [19]. The enzymatic hydrolysis was inactivated by boiling samples for 15 min in a water bath and then cooled in an ice bath. After hydrolysis the samples were centrifuged at 2254 gravities for 5 min to remove residual solids. Total reducing sugars (TRSs) were determined with dinitrosalicylic acid method (DNS) [20]. The amount of reducing sugars was estimated as mg equivalent glucose per g dry treated biomass.

2.5. Statistical design and optimization

A statistical 2^3-central composite design (CCD) was used for the design of experiments.

The CCD design was made up of 20 base runs (Table 2; 8 cube points, 6 center points in cube, 6 axial points, and 0 center points in axial), 1 single base block, all in duplicate, resulting in a total of 40 experiments. The three variables chosen were designated as X_1 (Temperature), X_2 (Time), X_3 (% H_2O_2). The model generated as a function of these variables on the predicted responses of cellulose content, hemicellulose solubilization, and lignin removal is a second-order polynomial and is represented as follows:

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

The predicted responses are designated as Y associated with each factor level combinations; α_0 to $\alpha_{2,3}$ are coefficients to be estimated from regression, they represent the linear, quadratic and cross-products of X_1, X_2, X_3 on the responses; X_1, X_2, X_3 are the factors. The statistical software package MINITAB 15 (PA, USA) was used for regression analysis of experimental data, plotting of response surfaces and to optimize the process parameters. The coefficients in the second-order polynomial (Eq. (3)) were calculated by multiple regression analysis, based on the experimentally obtained data, and then the predicted responses were obtained using Eq. (3). The order in which the experiments were carried out was randomized and all the experimental runs were carried out by a single operator to minimize block effect.

3. Results and discussion

3.1. Composition of the solid fraction

The APO pretreatment was aimed to fractionate the wood biomass into a solid fraction containing as much polysaccharides (importantly as cellulose) and as less lignin as possible; and a liquid fraction containing solubilized hemicellulose the best preserved as possible.

Lime consumption increased with increasing temperature. The average lime consumption was 0.10 g $Ca(OH)_2$ g^{-1} dry biomass (Table 2); which means that 3 g lime loading should be adequate to cause appreciable delignification. This value for specific lime consumption agrees with other studies on lime pretreatment of some lignocellulosic biomass [13,21].

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. The recalcitrant nature is also reflected in this work. However, subjecting the substrate to some pretreatment variations could increase its suitability for efficient enzymatic hydrolysis.

Percentage of dry matter recovered in the filtration cake ranged from 84% to 95%. High dry matter recovery corresponded to very low lignin removal. For example, pretreatment 9 with a dry matter yield of 95% showed only 4% lignin removal while pretreatment 3 with dry matter yield of 86% showed 13% lignin removal (Table 2). Cellulose recovery in the solid residues with an average value of 102% (pretreatments 1–20) proved the ability of the studied process for removing hemicellulose with negligible cellulose degradation. The joint contributions of cellulose and lignin recoveries accounted for the high dry matter yields in the solid residues. Hemicellulose recovery varied with all the conditions. It can be noted that the conditions of pretreatment 15 (150 °C, 40 min, 1%H_2O_2) corresponded to the maximum hemicellulose solubilization of up to 70%. Increased cellulose content in the pretreated solids ranged from 47% to 56% from the initial raw biomass of 46%. Cellulose content decreased at 120 °C (pretreatment 10) to 43% corresponding to a decrease in lignin removal. In this process, cellulose enrichment was due majorly to hemicellulose solubilization and a small percentage of lignin removal. The lignin removal was very low in all the conditions with the highest value of 16% (data not shown); this can be attributed to the high lignin content (29.9%) in the raw biomass. It was also noted that the materials pretreated at low temperatures (110.5–135 °C) appeared light brown in color while solids pretreated at 150 °C and 159.5 °C were deep brown in color.

Under these experimental conditions, it was revealed that more hemicellulose is solubilized than lignin removal. Even if little lignin removal was achieved in experiments, favorable effects for further enzymatic hydrolysis are expected to be caused by the alkaline peroxide oxidation process, including dramatic increase in the degree of hydration of the cellulose polymer [3].

3.2. Statistical analysis of results

Application of CCD on the pretreatment process generated the following second order polynomial equations for cellulose content, hemicellulose solubilization and lignin removal;

$$\text{Cellulose content } (\%\text{w/w}) = 88.274 + 0.1305X_1 - 0.879X_2 - 45.449X_3 - 0.003X_1^2 + 0.004X_2^2 - 3.307X_3^2 + 0.005X_1X_2 + 0.382X_1X_3 - 0.066X_2X_3 \quad (R^2 = 0.955) \tag{4}$$

$$\text{Hemicellulose } (\%\text{w/w}) = 771.776 - 10.324X_1 - 2.420X_2 + 45.736X_3 + 0.037X_1^2 - 0.006X_2^2 - 24.719X_3^2 + 0.016X_1X_2 - 0.079X_1X_3 + 0.507X_2X_3 \quad (R^2 = 0.973) \tag{5}$$

$$\text{Lignin removal } (\%\text{w/w}) = 57.845 - 1.030X_1 - 0.095X_2 + 22.566X_3 + 0.0033X_1^2 + 0.005X_2^2 - 20.2914X_3^2 - 0.002X_1X_2 + 0.203X_1X_3 + 0.126X_2X_3 \quad (R^2 = 0.840) \tag{6}$$

When the values from X_1 to X_3 were substituted in the above equations, the predicted responses were obtained. The predicted values were compared with the experimentally obtained values and the data were in close agreement (Table 3).

The statistical treatment combinations of the test variables corresponding to all combinations revealed reliable regression coefficients (values are not shown). The *p*-values were used as a tool to

Table 3
Experimental and predicted values for cellulose content, hemicellulose solubilization, and lignin removal in the solid fraction of APO treated sawdust.

APO condition	Cellulose content		Hemicellulose solubilization		Lignin removal	
	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	53.71	54.11	61.69	64.18	4.12	4.55
2	53.81	53.78	57.49	62.35	11.73	12.72
3	54.48	54.47	57.73	61.90	8.91	8.39
4	54.07	54.14	64.92	68.15	9.61	9.50
5	53.58	53.00	59.84	63.73	11.14	9.22
6	48.95	48.91	63.08	65.64	10.81	10.14
7	53.1	52.69	59.46	63.75	11.79	10.30
8	54.05	53.47	67.08	71.36	12.27	13.08
9	52.59	52.97	70.44	75.02	8.12	9.38
10	51.44	51.53	68.37	72.48	8.09	7.87
11	53.14	53.00	58.51	63.73	8.36	9.22
12	52.82	53.00	60.79	63.73	10.17	9.22
13	54.62	54.11	58.97	64.18	5.55	4.55
14	53.79	53.78	58.51	62.35	14.18	12.72
15	50.67	50.78	51.42	54.96	7.23	7.07
16	52.46	53.00	60.79	63.73	8.19	9.22
17	54.45	54.47	59.35	61.90	8.33	8.39
18	48.99	48.91	61.29	65.64	9.91	10.14
19	52.37	52.69	59.38	63.75	9.47	10.30
20	52.91	53.47	65.90	71.36	14.48	13.08
21	53.36	52.97	69.84	75.02	11.24	9.38

Table 4
Analysis of variance (ANOVA) for the polynomial models obtained from experimental design.

Cellulose content						
Source	DF	Seq SS	Adj SS	Adj MS	*F*	*P*
Regression	9	49.708	49.708	5.5231	25.87	0.0000
Linear	3	17.093	9.369	3.1230	14.63	0.0000
Square	3	12.858	6.620	2.2067	10.34	0.0020
Interaction	3	19.757	19.757	6.5860	30.84	0.0000
Residual error	11	2.348	2.348	0.2135		
Lack of fit	1	0.040	0.040	0.0400	0.17	0.6860
Pure error	10	2.308	2.308	0.2308		
Total	20	52.056				
Hemicellulose solubilization						
Regression	9	422.500	422.500	46.9444	44.39	0.000
Linear	3	186.298	179.448	59.8161	56.56	0.000
Square	3	190.355	203.789	67.9296	64.23	0.000
Interaction	3	45.847	45.847	15.2824	14.45	0.000
Residual error	11	11.634	11.634	1.0576		
Lack of fit	1	0.128	0.128	0.1283	0.11	0.745
Pure error	10	11.506	11.506	1.1506		
Total	20	434.134				
Lignin removal						
Regression	9	109.368	109.368	12.152	6.43	0.003
Linear	3	68.183	3.658	1.2190	0.65	0.602
Square	3	36.263	38.158	12.7194	6.74	0.008
Interaction	3	4.922	4.922	1.6408	0.87	0.486
Residual error	11	20.773	20.773	1.8884		
Lack of fit	1	0.026	0.026	0.0261	0.01	0.913
Pure error	10	20.747	20.747	2.0747		
Total	20	130.141				

check the significance of each of the variables as well as their interactive and quadratic effects. In general, smaller the value of $p(<0.05)$ and larger the magnitude of t-value, the more significant is the corresponding coefficient term. It was observed that time and H_2O_2 main effects, time and temperature quadratic effects, temperature interactions with time and H_2O_2 were statistically significant on cellulose content. All the main, quadratic, and interactive effects are significant on hemicellulose solubilization except temperature and H_2O_2 interaction. For lignin removal, only H_2O_2 quadratic effect was statistically significant. In addition, the multiple correlation coefficients (R^2) of the regression equations obtained were 0.9549 for cellulose content (R^2 adjusted = 0.9180), 0.9732 for hemicellulose solubilization (R^2 adjusted = 0.9513), and 0.8404 for lignin removal (R^2 adjusted = 0.7098). These values mean the models for the responses fitted well with the experimental data. The R^2-value for cellulose content implies that the sample variation of 95.5% is attributed to the factors, and also indicates that 4.5% of the total variation is not explained by the model. For hemicellulose solubilization 2.7% of the total variation is not explained by the model. R^2-value for lignin removal revealed that 16% of the total variation is not explained by the model.

The summary of analysis of variance (ANOVA) representing the results is discussed in Table 4. ANOVA is required to test the significance and adequacy of the models. The Fisher's variance ratio (F-value) is the ratio of the mean square owing to regression to the mean square owing to error [22]. It is the measure of variation in the data about the mean. The ANOVA for the three regression models indicates that models are very significant as evident from the calculated F-values and very low P-values ($P \leqslant 0.003$). Large F-value demonstrates that most of the variations in the responses can be explained by the regression model equations. The ANOVA table also shows a term for residual error, which measures the amount of variation in the response data left unexplained by the model. Thus, the form of the models chosen to explain the relationship between the factors and responses can be concluded to be correct.

Three-dimensional response surface curves were plotted to study the interactions between the various parameters in APO 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 (%w/w) cellulose content, hemicellulose solubilization, and lignin removal of the solid fraction are shown in Fig. 1A–C.

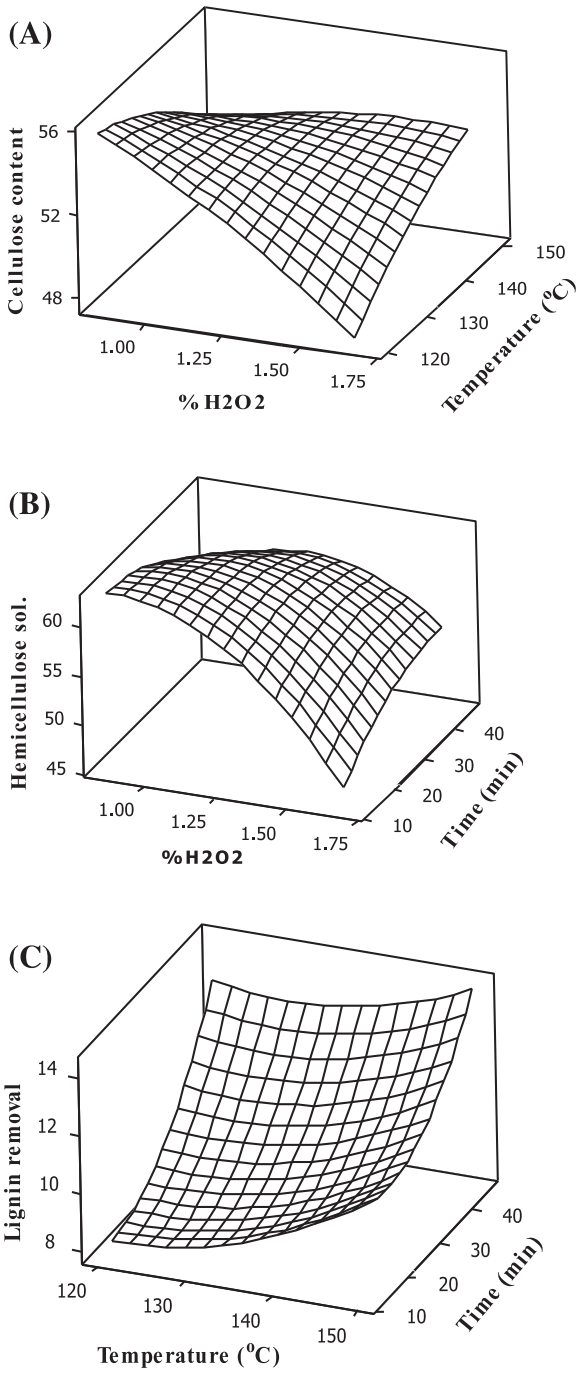

Fig. 1. Surface plots of the responses in the solid fraction of the pretreated sawdust. (A) Surface plot of cellulose content (%w/w) vs. temperature and $\%H_2O_2$; (B) surface plot of hemicellulose solubilization (%w/w) vs. time and $\%H_2O_2$; and (C) surface plot of lignin removal (%w/w) vs. time and temperature.

Fig. 1A–C shows the surface plots of the interactive effects of temperature, reaction time, % H_2O_2 on cellulose content, hemicellulose solubilization, and lignin removal to obtaining maximum responses. These plots were obtained by holding the third variable at mid point value. Maximum cellulose content should occur between 120–135 °C and 15–20 min or 140–150 °C and 40 min. Fig. 1A shows that maximum cellulose content is achievable at be-

Table 5
Predicted, experimental responses, and variations of pretreatments at the optimized conditions.

		A		B	C	B	E
		150 °C,1%H_2O_2, and 45 min		1%H_2O_2,10 bar 45 min	1%H_2O_2 soaking 10 min	10 bar 45 min	10 bar, 1%H_2O_2 soaking 10 min
	Raw biomass	Predicted responses	Experimental responses				
Solid fraction[a]							
Dry matter yield	100			79.78	88.78	83.23	82.35
Extractives	1.89			3.30	3.17	3.71	3.45
Cellulose	45.86	53.86	58.52	59.17	59.60	59.06	60.12
Hemicellulose[b]	20.31	70.00	71.84	7.28 (71.40)	4.77 (79.15)	6.56 (73.12)	4.88 (80.21)
Lignin[c]	29.90	11.00	9.64	28.54 (23.85)	30.8 (8.55)	28.84 (19.72)	30.03 (17.29)
Ash	2.04			1.71	1.66	1.83	1.52

[a] Solid compositions in (%w/w).
[b] Values in parentheses are hemicellulose solubilization.
[c] Values in parentheses are lignin removal.

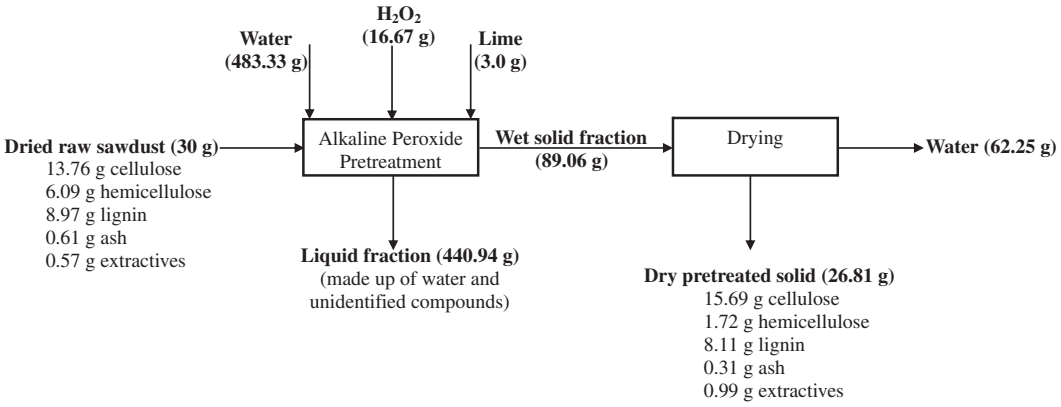

Fig. 2. Mass balance for the optimized alkaline peroxide oxidation pretreatment.

tween 1–1.25% H_2O_2 and 140–150 °C. Hemicellulose solubilization maximum response should also be between 1–1.25% H_2O_2 and 40 min (Fig. 1B). Maximum lignin removal will likely occur at 150 °C and 40 min (Fig. 1C).

3.3. Optimization of the pretreatment conditions

The optimal values of each factor to optimize the process responses were based on Multi-Objective Numerical Optimization. The model equations for the various responses (Eqs. (3)–(6)), and the response surfaces were utilized in determining the optimum APO conditions so as to obtain a solid fraction with high cellulose content, low lignin and hemicellulose. With all these constraints in mind, the optimum cumulative responses were obtained at 150 °C, 45 min, and 1%H_2O_2 (Table 5A). The predicted responses were 53.86% cellulose content (desirability = 0.9638), 70.00% hemicellulose solubilization (desirability = 0.8573), and 11.00% lignin removal (desirability = 0.9994). A validation of results from the models and regression equation was performed (Table 5B) and compared with the predicted values. The experimental and predicted responses were found to be in close agreement, thus confirming the optimization process. The mass balance for the optimized alkaline peroxide pretreatment conditions (150 °C, 1%H_2O_2, and 45 min) is presented in Fig. 2. The input stream includes the dried raw sawdust, H_2O_2, lime, and water. After pretreatment the output is divided into the wet pretreated solid and the filtrate (which includes water and unidentified compounds such as monosaccharides, degradation products, and gases). After pretreatment, the mass loss was due to solubilized components in the liquid fraction while after drying the wet biomass the mass loss was only due to water evaporating at the drying conditions. From the material balance, 3.19 g of biomass was lost from the initial 30 g dry raw material.

Further, variations at the optimized conditions were considered (Table 5, pretreatments B–E). The highest cellulose content (60%) was achieved in the solid fraction obtained at pretreatment E (APAWAO) with hemicellulose solubilization of 80% and 17% lignin removal. Lignin removal also increased to 24% with the addition of air at 10 bar air pressure and 45 min reaction time (pretreatment B). These variations indicated higher carbohydrate yields and more delignification by combining hydrogen peroxide and air at 10 bar pressure.

Lignin and acetyl groups in hemicellulose as well as crystallinity in cellulose and hemicellulose are significant challenges for cellulase enzymes to access lignocellulosic fiber matrix [23]. In addition, factors such as the composition of solid biomass, pH of medium, temperature of hydrolysis, the desired residence time, and enzyme activity may affect the enzymatic hydrolysis of lignocellulosic biomass to sugars. The effect of alkaline peroxide on pretreatment of sawdust at optimized conditions (150 °C and 1%H_2O_2 for 45 min) on 3-day enzymatic saccharification using a cocktail of two commercial enzyme preparations (cellulase and β-glucosidase) at specified conditions is presented in Fig. 3. Also, enzymatic hydrolysis results for variations considered at the optimized pretreatment conditions (WAO; 150 °C and 10 bar air pressure for 45 min, and APAWAO; 150 °C, 10 bar air pressure and 1%H_2O_2 for 45 min) are presented in Fig. 3. The TRS yields for the APO treatment (indicated as A in Fig. 3) varied between 58.59 mg g^{-1} dry biomass for 2 h and 177.89 mg g^{-1} for 72 h hydrolysis periods. The low sugar yields were expected as the pretreatment modified to a small extent

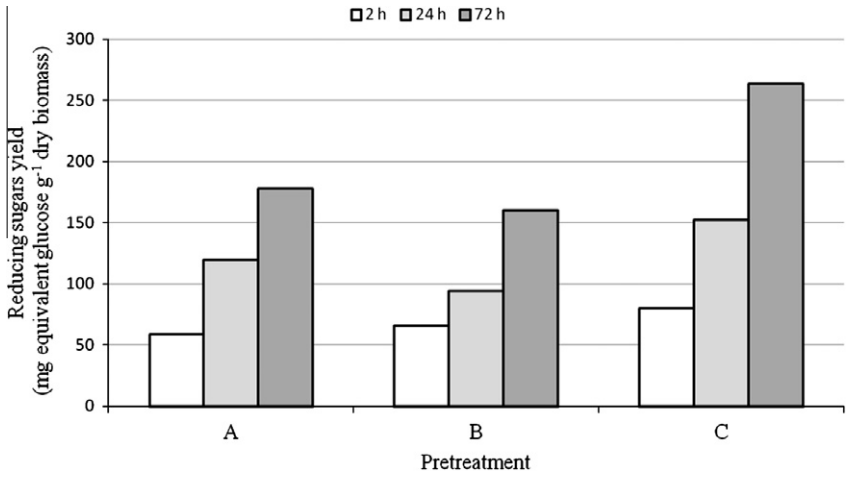

Fig. 3. Enzymatic hydrolysis results for alkaline peroxide oxidation (A), wet air oxidation (B), and alkaline peroxide assisted wet air oxidation treated wood waste (C).

the raw solid material due to the high lignin content. The WAO treatment (indicated as B in Fig. 3) produced a lower TRS yield (159.96 mg g^{-1}) at 72 h. However, alkaline peroxide assisted wet air oxidation treatment (APAWAO; indicated as C in Fig. 3) enhanced enzymatic saccharification up to 263.49 mg g^{-1} at the end of the third day. It follows that a combination of air and hydrogen peroxide as oxidizing agents increased delignification leading to increased enzymatic hydrolysis of treated biomass for the APAWAO conditions compared to the other oxidations (APO and WAO).

For industrial applications, a pretreatment agent must be effective, economical, safe, environmentally friendly, easy-to-use, and easy to recover [24]. Lime based oxidative pretreatment is said to meet these objectives [24]. Although the pretreatments were carried out at high operating temperatures because of the compositions of the raw material (high lignin material), short time duration (45 min), low average lime loading (0.1 g g^{-1} dry biomass) and low hydrogen peroxide concentration of 1%H_2O_2 (33.3 mL H_2O_2 L^{-1} water) are added advantages to this process. Lime pretreatment method is said to be a cost-effective pretreatment [25]. Future research will focus on investigating the pretreatment process at much lower temperatures and using different enzyme loadings on pretreatments.

4. Conclusions

The effects of the major operational variables (temperature, time, and %H_2O_2) involved in the alkaline peroxide oxidation pretreatment process of *V. paradoxa* sawdust, amenable to enzymatic hydrolysis, showed that extensive hemicellulose solubilization (up to 70%) can be achieved. Alkaline peroxide oxidation with lime as the alkaline agent caused only limited delignification (owing mainly to the recalcitrant nature of the high lignin wood material), whereas cellulose was almost quantitatively retained in the solid phase. An improvement in the lignin removal occurred with the addition of air. From the optimal pretreatment (150 °C, 1%H_2O_2, 45 min), the sugars yield in 72 h hydrolysis time, 25 FPU cellulase g^{-1} dry biomass, 12.5 IU g^{-1} dry biomass of β-glucosidase, 20 g L^{-1} substrate loading, 45 °C hydrolysis temperature was 177.89 mg equivalent glucose g^{-1} dry biomass. Enhanced pretreatment with the addition of 10 bar air pressure at the optimum conditions increased sugars yield to 263.49 mg equivalent glucose g^{-1} dry biomass. At the downstream processing, the lignin left undissolved can be useful in combustion for power generation.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fuel.2012.12.078.

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