

Utilization of lignocellulosic waste for ethanol production: Enzymatic digestibility and fermentation of pretreated shea tree sawdust

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(Received 9 July 2013 • accepted 22 January 2014)

Abstract—Enzymatic hydrolysis and fermentation methods were evaluated on alkaline peroxide pretreated shea tree sawdust conversion to ethanol. Optimum pretreatment conditions of 120 °C reaction temperature, 30 min reaction time, and 20 mL L⁻¹ of water hydrogen peroxide concentration (1%(v/v)H₂O₂) solubilized 679 g kg⁻¹ of hemicellulose and 172 g kg⁻¹ of lignin. 617 g kg⁻¹ cellulose was retained in the solid fraction. The maximum yield of reducing sugar with optimized enzyme loadings by two enzyme preparations (cellulase and β-glucosidase) was 165 g kg⁻¹ of dry biomass. The ethanol yield was 7.35 g L⁻¹ after 72 h incubation period under the following conditions: 2% cellulose loading, enzyme concentration was 25 FPU (g cellulose)⁻¹ loading, yeast inoculum was 10% (v/v), 32 °C, and pH 4.8. The pretreatments gave information about the hindrances caused by lignin presence in lignocellulosic materials and that hemicelluloses are better hydrolyzed than lignin, thereby enhancing enzymatic digestibility of the sawdust material.

Keywords: Alkaline Peroxide Oxidation, *Vitellaria paradoxa*, Pretreatment, Enzymatic Hydrolysis, Lignocellulosic Biomass

INTRODUCTION

Biomass is an interesting energy source for several reasons. The main reason is that bio-energy can contribute to sustainable development [1]. Resources are often locally available, and conversion into secondary energy carrier is feasible without high capital investment. Furthermore, since energy plantations may also create new employment opportunities in rural areas, it also contributes to the social aspect of sustainability. In addition, application of agro-industrial residues in bioprocesses not only provides alternative substrates but also helps solve their disposal problem [2]. As the transportation sector is practically entirely dependent on oil, and as it is responsible for half of the total CO₂ emission [3], increasing the market share of renewable bio-fuels, including fuel ethanol, is a topical issue around the world presently. Lignocellulosic wastes refer to plant biomass wastes that are composed of cellulose, hemicellulose, and lignin as well as other minor components. Both the cellulose and hemicellulose fractions are polymers of sugars, and thereby a potential source of fermentable sugars, or other processes that convert sugars into products. Hemicellulose can be readily hydrolyzed under mild acid or alkaline conditions. The cellulose fraction is more resistant and therefore requires more rigorous treatment. Lignocelluloses may be grouped into different categories such as wood residues (including sawdust and paper mill discards), grasses, waste paper, agricultural residues (including straw, stover, peelings, cobs, stalks, nutshells, non food seeds, bagasse, domestic wastes (lignocelluloses garbage and sewage), food industry residues, municipal solid wastes and the like. Lignocellulose is the most abundant renewable biomass with

a worldwide annual production of 1×10¹⁰ MT [4]. The shea tree is a woody lignocellulosic material, typically a savannah woodland tree species. *Vitellaria paradoxa* is a small to medium-sized tree 10-15 m tall with diameter ranging from 0.3 to 1 m. The tree is native to African countries such as Uganda, Benin, Cameroon, Ghana, Guinea, Nigeria, Senegal. Shea tree occurs on an estimated 1 million km² between western Senegal and northwestern Uganda. The species is found on various soil types but avoids land subject to flooding [5]. The exotic species is found in the Dominican Republic and Honduras. It is deciduous, its trunk makes excellent charcoal, and it is favored as a source of wood fuel.

Due to structural features, such as the presence of lignin, acetyl groups, and cellulose crystallinity, lignocellulosic biomass must be pretreated to enhance its enzymatic digestibility before microbial conversion into liquid fuels [6]. 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.

Alkaline peroxide oxidation (APO) pretreatment is known to decrystallize cellulose [7]. 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 [8]. 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 [7]. Extensive studies exist on the APO process pretreatment of agricultural residues [8-11]. Studies on alkaline peroxide treatment on woody biomass also exist in the literature [12,13]. Previously, we discovered that comparable pretreatment and enzymatic digestibility results were achievable at

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low temperatures than at high temperatures, thereby reducing the economy of the pretreatment process. It was also reported that a combination of air and hydrogen peroxide as oxidizing agents improved pretreatments than when only hydrogen peroxide was applied [12,13].

In this study, APO pretreatment of shea tree (*Vitellaria paradoxa*) sawdust was evaluated at lower temperatures without air pressure addition with respect to separating the material to its different components, enzymatic digestibility and fermentation of the pretreated solid fraction to ethanol.

MATERIALS AND METHODS

1. Design of Experiments

Raw material preparation from the field to the laboratory before compositional analysis has been described in earlier studies [12,13]. A statistical 2²-central composite design (CCD) was used for the design of experiments [14,15] (Table 1). The CCD design was made up of 13 base runs (four cube points, five center points in cube, four axial points, and zero center points in axial, as shown in Table 2), one single base block, all in duplicate, resulting in a total of 26 experiments. In our previous studies [12,13], we established optimum concentration of hydrogen peroxide needed to cause appreciable delignification for the shea tree sawdust to be 1% H₂O₂ (v/v), maximum lime loading was also established. As a result, two operating process parameters, reaction temperature and reaction time, were considered in this study. The objective was to evaluate the influence of pretreatment time, and temperature on the APO process such that the cellulose content, the hemicellulose solubilization, and deligni-

Table 1. Statistical 2²-central composite design for APO pretreatment

Factor	Low level	High level
Reaction temperature, X ₁ (°C)	90	120
Reaction time, X ₂ (min)	15	30

Table 2. Experimental design matrix for pretreatment, cellulose content, solubilization after biomass pretreatment and reducing sugar yields after enzymatic hydrolysis

Run	Temperature (°C)	Time (min)	Lime consumption ^a	Cellulose content ^a	Hemicellulose solubilization ^a	Lignin removal ^a	RS ^a
1	120	15	38.9±5.4	590.3±15.3	708.0±25.6	140.9±10.1	81.4±6.1
2	105	11.89	31.1±3.8	582.8±11.5	656.5±19.1	71.4±13.2	128.5±9.1
3	90	30	27.3±2.1	600.8±15.4	587.0±20.4	134.3±14.9	91.9±3.7
4	105	22.5	33.7±3.4	606.4±13.1	609.2±14.6	146.6±17.7	121.8±12.1
5	105	33.11	29.4±5.2	555.9±19.0	585.2±18.0	91.7±12.0	124.4±6.5
6	105	22.5	27.7±4.9	582.0±18.1	625.3±24.4	142.9±15.6	85.8±10.0
7	105	22.5	24.6±3.8	585.9±19.4	629.1±23.3	168.4±4.8	121.3±13.0
8	90	15	25.4±5.1	591.8±14.0	594.8±24.2	128.3±9.1	87.7±9.8
9	83.79	22.5	27.0±5.9	566.9±17.6	581.0±21.0	126.5±4.4	102.8±12.1
10	105	22.5	25.1±4.5	562.4±11.3	586.7±26.7	49.6±5.7	120.8±24.3
11	120	30	39.0±4.8	611.2±33.2	679.3±32.7	172.0±27.4	146.0±15.0
12	105	22.5	27.1±5.7	580.3±14.8	622.6±14.0	135.9±11.0	98.9±11.2
13	126.21	22.5	39.5±2.4	589.8±11.6	685.8±19.6	62.7±26.7	128.0±10.0

^aIn g kg⁻¹ dry biomass. Data are means of two replicates

fication will enhance enzymatic hydrolysis and production of ethanol from reducing sugars released during enzymatic hydrolysis. They were chosen for study as these parameters can influence the fractionation of the solid material. Hydrogen peroxide concentration and lime loading were maintained at constant levels throughout the pretreatments. MINITAB 15 statistical software (PA, USA) was used for the design of the pretreatments. 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. Each experiment in this study was replicated twice; reported results indicate the mean values of the replicated experiments.

2. Experimental Set Up

The pretreatment was carried out in a 1.8 L volume Parr reactor (Model-4578, Floor stand HP/HT, Parr Instruments, IL, USA). The reactor was fitted with double six-blade turbine impellers with an external heating embedded in a jacket. Internal stainless steel loops provided cooling in the reactor, a solenoid valve adjusted the flow of water through the internal coils. 25 g of dry substrate was mixed with 500 mL distilled water containing 1% H₂O₂ (hydrogen peroxide content was 20 mL L⁻¹ of water) solution and adjusted to pH 11.5 with 3.2 g of Ca(OH)₂ (lime). Slurries were pretreated at different temperatures, and at different time intervals (Table 2).

The reaction was controlled by a Parr PID temperature controller model 4857. Temperature in the reactor was regulated within ±2 °C of the set point values with constant stirring at 200 revolution per minute. Each reaction was terminated by running cold water through the internal loops. After the specified reaction time, the reactor and slurry were allowed to cool to ambient temperature. The content of the reactor was transferred into a 1 L beaker. The biomass slurry was neutralized with 5 M HCl to determine the amount of unreacted lime. Using this value and the known initial quantity of lime, lime consumption in g kg⁻¹ dry biomass for each reaction time was determined [16,17] (Table 2). The pretreated slurry was separated into the solid and liquid fractions by vacuum filtration, and the neutralized solid fraction was washed with water. A portion of the treated wet solid fraction was taken for compositional

Table 3. Chemical composition of raw and pretreated sawdust in g kg⁻¹ dry biomass

	Dry biomass yield	Extractives	Cellulose	Hemicellulose	Lignin	Ash
Raw biomass	1000	18.9±1.7	458.6±9.2	203.1±11.5	299.0±13.2	20.4±3.1
Run order						
1	859.4±56.3	26.1±2.1	590.3±10.7 (1106.2) ^b	69.0±7.2(292.0) ^b	298.9±13.7(859.1) ^b	15.7±2.3
2	933.9±45.3	30.6±2.5	582.8±17.5 (1186.8)	74.7±7.4(343.5)	297.3±11.7(928.6)	14.6±1.6
3	927.8±48.2	14.1±4.2	600.6±12.7 (1215.1)	90.4±2.4(413.0)	279.0±24.7(865.7)	15.6±1.7
4	940.5±36.2	21.9±2.8	606.4±21.8 (1243.6)	84.4±7.8(390.8)	273.3±19.4(853.4)	15.0±1.6
5	933.0±11.7	47.4±2.3	555.9±16.4 (1131.0)	90.3±10.5(414.8)	291.1±1.7(908.3)	15.3±2.8
6	881.9±27.6	27.2±6.4	582.0±15.6 (1119.2)	86.3±7.9(374.7)	290.6±15.0(857.1)	13.9±2.1
7	872.8±40.9	26.5±3.1	585.9±12.6 (1115.1)	86.3±8.1(370.9)	284.9±12.3(831.6)	16.4±2.1
8	937.2±49.1	18.9±2.3	591.8±11.0 (1209.4)	87.8±7.6(405.2)	278.1±19.4(871.7)	19.4±1.8
9	930.1±30.7	44.6±1.0	566.9±12.5 (1149.8)	91.5±4.8(419.0)	280.8±19.5(873.5)	16.2±3.3
10	941.0±46.5	31.8±1.7	562.4±12.6 (1154.0)	89.2±8.1(413.3)	302.0±9.6(950.4)	14.6±4.2
11	900.9±45.3	24.8±2.4	611.2±14.8 (1200.7)	72.3±10.6(320.7)	274.8±29.4(828.0)	16.9±3.0
12	893.4±11.9	29.5±3.5	580.3±11.4 (1130.5)	85.8±8.1(377.4)	289.2±18.7(864.1)	15.2±3.4
13	942.7±32.8	31.1±4.0	589.8±9.2 (1212.4)	67.7±10.3(314.2)	297.3±11.0(937.3)	14.1±4.0

^bRecovery of the components in parentheses. Data are means of two replicates

analysis while the remaining part was stored frozen for later enzymatic processing and fermentation.

3. Analysis of the Raw and Pretreated Materials

Compositional analysis on the raw and pretreated samples is as previously described by Ayeni et al. [12,13]. The dry solid content was analyzed by a convection oven. Extractives were determined by means of the Soxhlet extractor using 300 mL acetone as solvent on 5 g of dry biomass 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 and further dried at 105 °C in a convection oven. The extractive content was calculated as the difference in weight between the raw and extracted material [12,13,18-20]. Mineral components were determined by ashing at 575 °C for 6 h. The hemicellulose content was determined by placing 1 g of dried biomass from the extractive analysis into a 250 mL Erlenmeyer flask and then 150 mL of 500 mol m⁻³ NaOH solution was added. The mixture was boiled for 3 hours and 30 minutes with distilled water. The residue was dried to a constant weight at 105 °C and later cooled in a desiccator and weighed. The difference between the sample weight before and after this treatment is the hemicellulose [19,20]. Lignin composition was determined by weighing into glass test tubes 300 mg of dry extracted biomass and adding 3 mL of 72% H₂SO₄. 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 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 hydrolyzates were cooled to room temperature and filtered through vacuum using a filtering crucible (Borosil 3206012, Grade 4 (pore size: 5-15 µm), 50 mL capacity). 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 [21]. The cellulose content was calculated by difference, assuming that extractives, hemicellu-

lose, lignin, ash, and cellulose are the only components of the entire biomass [18-20].

4. Recovery of Components

The recovery of components was evaluated to find the amount of lignin removed, hemicellulose solubilized, and the cellulose content of the pretreated solids as shown in Tables 2 and 3. An efficient oxidative pretreatment is to remove lignin while reducing cellulose degradation. The more the lignin removal the better the cellulose matrix is exposed to enzymatic hydrolysis. The recovery of components was calculated according to the following equation [22]:

$$Y_i = \frac{C_i Y_t}{C_{i0}} \quad (1)$$

where i is the component (lignin, hemicellulose, cellulose), Y_t is the recovery of component at time t (g kg⁻¹ of component i in raw biomass), C_i is component i in time t (g kg⁻¹ of residual biomass), Y_t is the total solids recovered at time t (g kg⁻¹ of raw biomass), C_{i0} is the component i content at time 0 (g kg⁻¹ of raw biomass).

5. Enzymatic Hydrolysis of Washed Pretreated and Untreated Materials

The pretreated and untreated washed solid fractions were hydrolyzed by enzymes to determine the efficiency of substrate conversion. Enzymatic conversion was performed at 2% dry substrate (20 g kg⁻¹ dry biomass content). Sodium citrate buffer (5 mL, 0.1 M, pH 4.8), 0.04 mL tetracycline (10 mg mL⁻¹ in 70% ethanol) were added to the wet materials in 30 mL culture tubes. A commercial preparation of *Trichoderma reesei* cellulase enzyme system (EC 3.2.1.4) with an activity of 57.8 filter paper unit (FPU) mL⁻¹ (kindly provided by M/s Zytex, Mumbai, India) and β-glucosidase (EC 3.2.1.21) with an activity of 10 international unit mg⁻¹ solid were added at a loading of 25 filter paper unit (g dry biomass)⁻¹ (the dry biomass as the addition of cellulose and hemicellulose contents in treated materials) and 12.5 international unit (g dry biomass)⁻¹, respectively. An appropriate volume of distilled water was added to bring the total volume to 10 mL. The progress of the reaction was measured by removing 0.5 mL aliquot at hydrolysis time intervals of 2, 24, and 72 h. Experi-

ments were conducted at 50 °C in a shaking incubator at 130 revolution minute [23]. To stop the hydrolysis, the samples were boiled for 15 min 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 3,5, dinitrosalicylic acid method [24]. The amount of reducing sugars (RS) was calculated as follows [25]:

$$\begin{aligned} &\text{Reducing sugar yield from enzymatic hydrolysis (g kg}^{-1}\text{ dry biomass)} \\ &= \frac{\text{amount of reducing sugar produced after hydrolysis}}{\text{amount of dry biomass}} \quad (2) \end{aligned}$$

Furthermore, enzymatic digestibility was considered at increased dry biomass loading of 3, 4, and 5% with corresponding increase in enzyme loadings for the pretreatment that resulted in highest reducing sugar yield out of the initial 13 experimental runs. Enzymatic conversions with and without β -glucosidase supplements and 45 °C hydrolysis temperature for four days were investigated. The effect of enzyme loadings on treated solids was also evaluated.

6. Simultaneous Saccharification and Fermentation of Pretreated Solids

Simultaneous saccharification and fermentation (SSF) method was used to investigate the conversion of treated solids to ethanol [23]. Biomass cellulose loadings investigated were 2% (5.02 g of treated sample) and 3% (7.53 g of treated sample) of dry solids for a total fermentation mixture of 50 g. The enzyme loading was kept at 25 FPU (g cellulose)⁻¹ loading. *Saccharomyces cerevisiae* was kindly provided by Puri Power and Sugar Ltd., Umrer District, Nagpur, India. The inoculum was developed on MYPD (malt wxtract, yeast extract, peptone, dextrose) medium containing the following ingredients (g L⁻¹): malt extract, 3.0; yeast extract, 3.0; peptone, 5.0; glucose, 10.0 (medium was adjusted to pH 4.8±0.2 with citrate buffer). The medium components were initially sterilized by steam autoclaving at 121 °C for 30 min. Inoculation flasks were incubated at 30±2.0 °C for 24 h under shaking conditions (130 revolution per minute). Cells were grown to an optical density (OD₆₀₀) at 0.6 [23]. The fermentation was carried out in 250 mL Erlenmeyer flasks with the incubator shaker at 30±2.0 °C for 72 h. At the end of the fermentation period 5 mL of mixture was removed and centrifugation was performed at 4,500 revolution per minute for 5 min. Ethanol analysis was carried out from the absorbance of the sample at 590 nm using the dichromate assay method [26].

RESULTS AND DISCUSSION

1. Effect of Lime on Pretreatment

Nagwani [27] reported that time and temperature had the greatest impact on biomass digestibility for lime pretreated biomass. In general, as shown in Fig. 1, the amount of lime consumed increased with increasing temperature. Lime consumption ranged from 25 to 40 g lime consumed kg⁻¹ raw biomass. The specific lime consumption trend agrees with other studies on lime pretreated biomass [16, 17]. Chang et al. [17] established that lime consumption increased with temperature, but maximum lime consumed did not exceed 0.1 g Ca(OH)₂ g⁻¹ dry biomass. Lignin removal increased between 100 °C and 110 °C and decreased as the temperature increased. More of the lime was consumed at high temperatures, which corresponded to increasing lignin removal. The highest lignin removal obtained

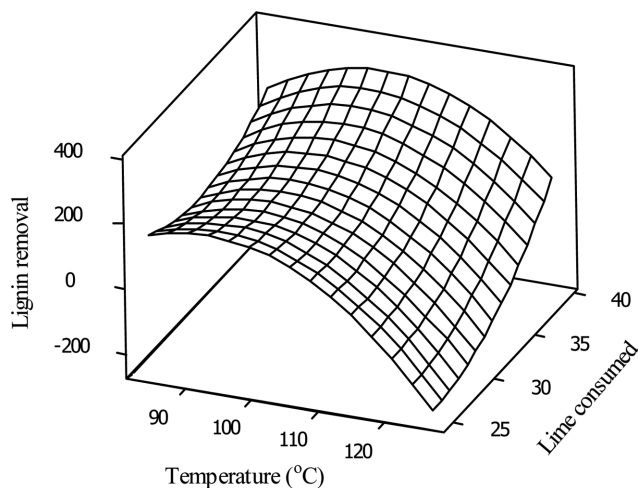


Fig. 1. Surface plot of lignin removal (g kg⁻¹) vs. Lime consumed (g kg⁻¹) and temperature.

was 172 g kg⁻¹ corresponding to about 40 g of lime consumed kg⁻¹ dry biomass (run 11).

2. Effect of APO Pretreatment on the Composition of the Solid Material

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. Percentage of dry solid recovered in the pretreated solid ranged from 860 to 943 g kg⁻¹. High dry biomass recovery corresponded to very low lignin removal. Cellulose recovery in the solid residues proved the ability of the studied process for removing hemicellulose with negligible cellulose degradation. Pretreatment 4 had the highest cellulose recovery as shown in Table 3. The joint contributions of cellulose and lignin recoveries accounted for the high dry biomass yields in the solid residues. Hemicellulose recovery varied with all the conditions. The experimental run 1 (120 °C and 15 min) corresponded to the maximum hemicellulose solubilization of up to 708 g kg⁻¹ (Table 2). Increased cellulose content in the pretreated solids ranged from 556 to 606 g kg⁻¹ from the initial raw biomass of 459 g kg⁻¹.

In the APO process, cellulose enrichment was due primarily to hemicellulose solubilization and a small percentage of lignin removal. The lignin removal was very low in all the conditions and was statistically confirmed using MINITAB 15 software, with the highest value of 172 g kg⁻¹ (Table 2); this can be attributed to the high lignin content (299 g kg⁻¹) in the raw biomass. The pretreatments resulted in fewer modifications of the compositions of the solid fraction.

Under these experimental conditions, it was revealed that more hemicellulose is solubilized than lignin removal. Silanikove [28] reported that CaO-H₂O₂ and NaOH-H₂O₂ treatments of cotton straw at room temperature for 25 h caused 500-580 g kg⁻¹ lignin removal. This higher value of lignin removal compared to this study may be attributed to the low lignin content in the raw cotton straw as well as the different operating conditions, which increased the solubilization of the lignin from the solid fraction. For oak shavings, a wood residue, it was shown that solubilization occurred under strong mechanical agitation and harsh pretreatment conditions [7]. However, the small lignin removal achieved in the experiments investigated can

cause favorable effects for further enzymatic hydrolysis made possible by the alkaline peroxide oxidation process, including dramatic increase in the degree of hydration of the cellulose polymer [7].

3. Effect of APO on the Enzymatic Digestibility of Treated and Untreated Solid Material

Table 2 shows the RS yields (g kg^{-1}) after hydrolysis of pretreated sawdust at 50°C and 72 h hydrolysis time for the 13 base runs. Reducing sugar yield of pretreated substrate was between 81.36 and $146.00 \text{ g equivalent glucose per kg of dry solid}$ for the 72 h hydrolysis time. Note that under the operating conditions, maximum RS yield obtained was 146.00 g kg^{-1} dry biomass (run 11) (120°C , $1\% \text{H}_2\text{O}_2$ and 30 min). This was expected as this pretreatment modified to a small extent the raw solid material. Lignin content in lignocellulosic biomass has a great influence on digestibility of the material [29-31]. Saha and Cotta [10] using $\text{NaOH-H}_2\text{O}_2$ treatment on rice hulls (with lignin content of 187 g kg^{-1}) at room temperature achieved total sugars yield of 353 g kg^{-1} dry biomass in 120 h pretreatment (74% conversion of treated substrate). The enzymatic digestibility conditions in this study did not produce appreciable sugar yields. The operating conditions needed to be altered for better enzymatic hydrolysis performance.

It is not possible to define a single optimum for enzymatic hydrolysis since this may shift depending on factors such as dry solid content, pH, temperature, the desired residence time, and enzyme activity. Since the enzymes are inhibited by the end products, the build-up of any of these products negatively affects cellulose hydrolysis. The concentration of cellulase enzyme complex has a high impact on the conversion of the cellulose. The maximum cellulase activity for most fungal derived cellulases and β -glucosidase occurs at $50 \pm 5^\circ\text{C}$ and a pH of 4.0-5.0 [32]. At lower temperatures, the hydrolysis rate per unit of active enzyme is slower, but so is enzyme denaturation [6]. The optimum temperature and pH is not only a function of the raw material and the enzyme source, but is also highly dependent on the hydrolysis time. The optimal conditions change with the hydrolysis residence time [33] and are also dependent on the source of the enzymes. The effects of substrate concentration variation ($20, 30, 40,$ and 50 g L^{-1}) with and without supplemental β -glucosidase, hydrolysis temperature (45°C), enzyme loadings as well as hydrolysis time (4-day) on digestibility were investigated on the pretreatment conditions 120°C , $1\% \text{H}_2\text{O}_2$, and 30 min (enzyme hydrolysis conditions: 20 g L^{-1} (25 FPU, 15 IU), 30 g L^{-1} (37.5 FPU, 22.5 IU), 40 g L^{-1} (50 FPU, 30 IU), 50 g L^{-1} (62.5 FPU cellulase ($\text{g dry biomass}^{-1}$), 37.5 IU β -glucosidase ($\text{g dry biomass}^{-1}$); 45°C hydrolysis temperature, pH 4.8).

Reducing sugar yields without β -glucosidase supplement were higher than when the enzyme was added, as shown in Figs. 2 and 3. The highest sugar yield under these conditions was 154 g kg^{-1} dry biomass at 72 h hydrolysis time when the highest substrate concentration of 50 g kg^{-1} was used. However, at 40 g kg^{-1} substrate loading under the same conditions, sugar yield was 148 g kg^{-1} dry biomass, which was 4.0% lower than higher substrate loading of 50 g kg^{-1} . Sugar yields decreased after 72 h for all substrate concentrations considered without β -glucosidase supplement but were likely to increase after the third day for 30 and 50 g kg^{-1} substrate loadings with β -glucosidase supplement.

Furthermore, enzyme loadings, hydrolysis of untreated and washed biomass were also evaluated. The untreated solid material was used

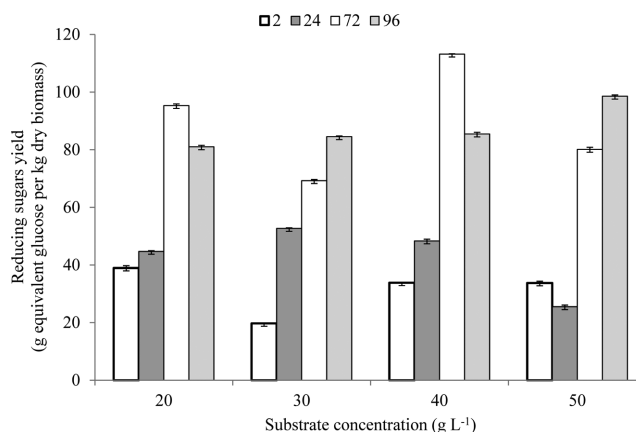


Fig. 2. 4-d Effect of time and substrate concentration on sugars yield with supplemental β -glucosidase; Pretreatment conditions: 120°C , $1\% \text{H}_2\text{O}_2$, and 30 min.

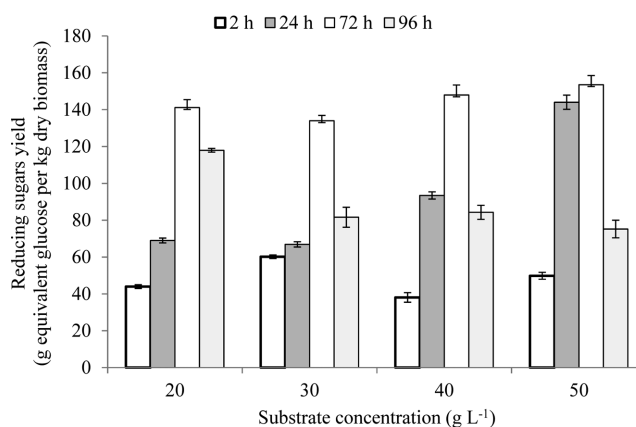


Fig. 3. 4-d Effect of time and substrate concentration on sugars yield with no supplemental β -glucosidase; Pretreatment conditions: 120°C , $1\% \text{H}_2\text{O}_2$, and 30 min.

as the control for comparing the enzymatic digestibility of the treated sawdust. The 4-day reducing sugar yields of untreated and pretreated sawdust were plotted against substrate concentrations as shown in Fig. 4 with no supplemental β -glucosidase. Concentrations in

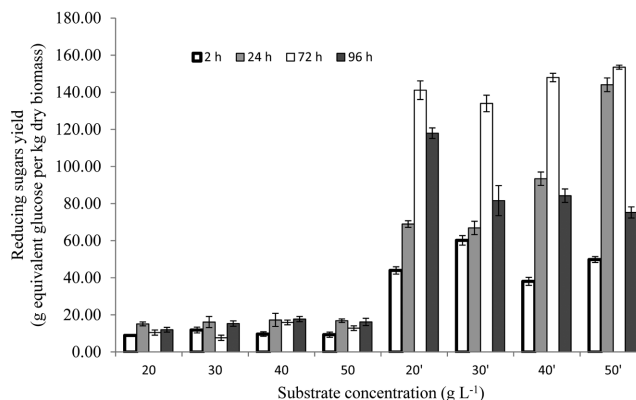


Fig. 4. 4-d Effect of time and substrate concentration on sugars yield for untreated and treated biomass with no supplemental β -glucosidase.

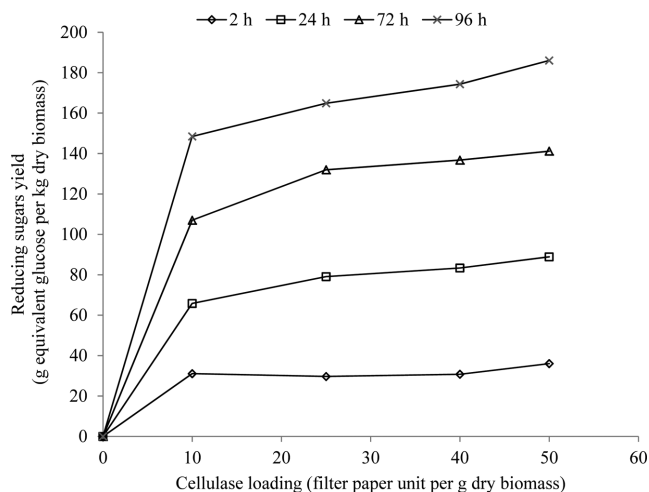


Fig. 5. 4-d Effect of enzyme loading on sugar yields. Pretreatment conditions: 120 °C, 1% H₂O₂, and 30 min. Enzyme hydrolysis conditions: 5 UI β -glucosidase per g dry biomass, 45 °C hydrolysis temperature, pH 4.8, 40 g L⁻¹ substrate concentration.

prime notation indicate enzymatic hydrolysis of treated samples. It can be noted from Fig. 4 that it was necessary to treat the raw material before enzymatic saccharification. Pretreatment is said to cause a disruption in the lignocellulosic matrix, thereby making the enzymes more accessible to substrates. The sugar yields of the pretreated sawdust are significantly higher than untreated sawdust. Results showed treated biomass maximum reducing sugars concentration of 148.0 g kg⁻¹ to untreated material of 10.5 g kg⁻¹ for the 40 g L⁻¹ substrate loading. This was a 14-fold increase in reducing sugars produced from the treated to the untreated biomass.

The enzyme loading study as shown in Fig. 5 was based on enzyme loadings of 10, 25, 40, and 50 filter paper unit per g dry biomass for 4-day hydrolysis time, 40 g L⁻¹ substrate concentration and 45 °C hydrolysis temperature. First and foremost, higher reducing sugars yields were obtained at higher enzyme loadings, which increased steadily to the 96 h period. Long incubation time with lower enzyme hydrolysis temperature was reported to increase the enzymatic saccharification of alkaline peroxide pretreated rice hulls [10]. From the results of this study, 25 FPU (g dry biomass)⁻¹ loading should be appropriate for the pretreatment conditions considered with reducing sugars yield of 165 g kg⁻¹ dry biomass. Beyond this enzyme loading, the enzymatic hydrolysis becomes uneconomical as reducing sugar yields did not change significantly. Cellulase loadings greater than 25 FPU (g dry biomass)⁻¹ may have caused the cellulose sites to be saturated by the enzymes. Sharma et al. [34] also reported that at optimum enzyme loading of 25 FPU (g dry biomass)⁻¹ on steam explosion pretreated sunflower stalk, there was an increase in the reducing sugar yields, beyond which saccharification decreased. Therefore, a cellulase loading of 25 FPU (g dry biomass)⁻¹ should be sufficient from an economic viewpoint. The 2% effective cellulose loading (5.02 g wet biomass) for the SSF conditions produced an ethanol yield of 7.35 g L⁻¹ with theoretical ethanol yield of 24.53%. On the other hand, 3% cellulose loading (7.53 g wet biomass) produced an ethanol yield of 7.79 g L⁻¹ with theoretical ethanol yield of 17.33%. These results suggest that the

lower ethanol yields from treated biomass resulted because a smaller portion of cellulose was digested by cellulase. However, a higher theoretical ethanol yield was obtained for 2% cellulose loading. The lower theoretical ethanol yield from the 3% cellulose loading may be due to consumption of accumulated ethanol by yeast [35]. Ramon-Portugal et al. [36] reported that when the ethanol accumulates in the medium, the microbial population is adapted to consume simultaneously sugar and ethanol.

CONCLUSION

The results of this study allowed the evaluation of alkaline peroxide oxidation pretreatment of sawdust before enzymatic hydrolysis and fermentation. The optimal pretreatment conditions tested for enzymatic hydrolysis were 120 °C for 30 min reaction time with the addition of 1% H₂O₂. From the optimal pretreatment, the best sugar yield in 96 h hydrolysis time, 25 filter paper unit g⁻¹ dry biomass, 40 g L⁻¹ substrate loading, 45 °C hydrolysis temperature was 165 g kg⁻¹. In our opinion, the high lignin content of the wood residue may have caused the low effect of APO on the enzymatic hydrolysis of the material, even with all the varied enzymatic hydrolysis conditions investigated. The lignin fraction of substrate remained largely un-dissolved. The un-dissolved lignin fraction affected the theoretical ethanol yield (which was maximum at 24.53% for 2% cellulose loading) of the fermentation process. However, the un-dissolved lignin can be available for energy production by combustion. More severe pretreatment conditions need to be further investigated in the future.

ACKNOWLEDGEMENTS

The author (A.O.A) is grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India and the Academy of Sciences for the Developing World (TAS), Italy, for the award of CSIR-TWAS fellowship for Research and Advanced Training tenable at National Environmental Engineering Research Institute (NEERI), Nagpur, India. The Nigerian Conservation Foundation and Chevron Nigeria Limited are appreciated for the Chief S.L. Edu research grant award. Also appreciated is the management of Covenant University, Ota, Nigeria for granting a one-year leave for this study.

NOMENCLATURE

APO : alkaline peroxide oxidation
 CCD : central composite design
 EC : enzyme classification
 FPU : filter paper unit
 IU : international unit
 OD : optical density
 PID : proportional-integral-derivative
 RS : reducing sugars
 SSF : simultaneous saccharification and fermentation

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