IOP Conference Series: Earth and Environmental Science

PAPER • **OPEN ACCESS**

Investigating the endothermic nature of the reactions of hybrid hydroxideperoxide with lignin in corn stover and napier grass for simple sugar production

To cite this article: S E Sanni *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **331** 012005

INVESTIGATING THE ENDOTHERMIC NATURE OF THE REACTIONS OF HYBRID HYDROXIDE-PEROXIDE WITH LIGNIN IN CORN STOVER AND NAPIER GRASS FOR SIMPLE SUGAR PRODUCTION

S E Sanni[1*], J A Omoleye[1], E M Emetere[2,3] and E E Okoro[4]

[1] Chemical Engineering Department, Covenant University Ota, Nigeria
[2] Physic Department, Covenant University Ota, Nigeria
[3] Department of Mechanical Engineering and Science, University of Johannesburg, APK, South Africa.
[4] Petroleum Engineering Department, Covenant University Ota, Nigeria
E-mail: adexz3000@yahoo.com

Abstract. Napier grass and Corn Stover samples were collected as waste materials for sugar production. The samples were pretreated and hydrolyzed using hydrogen peroxide and *Trichoderma ressei* cellulase respectively. Chemical kinetics of the delignification process shows that the reduction reactions of lignin during pretreatment are dependent on the nature of raw material, temperature, concentration of the hydrogen peroxide and their activation energies. The calculated activation energies show that the reactions of lignin in both biomasses are though endothermic, but less energy intensive and more economically viable for Napier grass when compared to corn Stover. Based on the results, in order to obtain higher sugar yield from Napier grass and corn stover under the investigated conditions, both samples should be processed at 105 ^{0}C for 96 hours using 0.3 M and 0.1M hydrogen peroxide concentrations respectively.

Key words: Biomass; Delignification; Kinetics; hydrogen peroxide pretreatment; Alkali pretreatment

1. Introduction

Lignocellulosic biomasses are currently the most preferable sources of reducing sugars. Lignocellulose consists of cellulose, hemicellulose and lignin, [1-3]. The sugar chains in cellulose can be hydrolyzed to their monomeric forms [4]. Cellulose can be chemically split into, glucose units by treating it with concentrated acids [5, 6] or enzymes. Hemicellulose is a copolymer of different amounts of several saccharide molecules [7]. It is less chemically and thermally stable compared to cellulose because of its non-crystalline nature [8]. Lignin is a polymer linked by phenylpropane units [9]. It offers resistance to degradation and provides hydrolytic stability and structural robustness in plants [10-18] hence, its removal is pertinent for easy biomass digestion by enzymes/acids during hydrolysis. In this paper, chemical pretreatments and enzymatic hydrolysis of Napier grass and corn stover were investigated, in order to ascertain the endothermic nature of lignin reactions with hybrid-hydroxide peroxide mix. This is because, oxidizing agents, such as hydrogen peroxide and ozone have been reported to have high delignification efficiencies [19-20]. Also, alkali pretreatment gives delignification efficiencies of 60-80% [4]. Till date, besides the proposal by Sanni et al. [21] concerning the reaction between lignin in Napier grass and NaOH, no work has considered the nature of the reactions of hybrid NaOH-H_2O_2 mix with lignin in biomass.

2. Materials and Equipment

30% pure H_2O_2 manufactured by Sigma Aldrich, sodium hydroxide (500g NaOH) manufactured by J.T Baker Inc. Philipsburg USA**,** sulphuric acid (2.5L H_2SO_4 of 98% purity, manufactured by J N Chemicals), distilled water (99% pure) produced by all glassware distiller Stwart W400 and *Trichoderma ressei* (Fungi)

Digital magnetic hotplate stirrer: MS7-H550-S LED (manufactured by SCILOGEX), autoclave with capacity 75-200L, string IIB Bench top steam sterilizer (made by Celitron –Azteca), Shaking incubator: VS-8480sr-I with D.C motor and PID controller (manufactured by Vision Scientific Co. Ltd.), petri dishes (manufacturer; J Sill Borosilicate), Oven Vs-1202d3 (Vision Scientific Co. Ltd., material of construction is steel with powder coating), UV mass spectrophotometer: 7310 visible Scanning Spectrophotometer fitted with 10 x 10 mm cuvette holder, 320 - 1000nm wavelength (Manufactured by Jenway), pH meter: Orion^{TM} Dual Star^{TM} pH, ISE Mv, ORP with temperature dual channel Bench top meter (manufactured by Thermo Fisher Scientific), soxhlet extractor (Amfield UK), Furnace, with maximum temp. of 1100-1300^{0}C and 5 - 23L chamber volume (made by Carbolite Gero), vacuum filtration setup comprising of vacuum pump, filter flask and Buchner funnel, electronic weighing balance (Manufactured by Swastic Systems and Services, Shahdara, Delhi, India) and water bath: 12 L capacity, 120V/60Hz, ELED 2837 (made by Thermo Scientific).

3. Method

Three months old Corn stover and Napier grass were both harvested from Adewale farm field in Atan, Ota, Ogun state, Nigeria. After being washed and sun dried for 3 days, they were crushed, milled and stored in air tight containers for 2 days in readiness for biomass pretreatment and hydrolysis.

3.1. Preparation of Napier Grass and Corn Stover Biomasses

Napier grass and Corn stover-impurities such as leaves/grass, sand, stones and dirt) were sorted out. The raw biomasses were then weighed and washed; the waters were kept to determine the presence of lignin in water from the washed biomasses. The biomass samples were air-dried and subsequently oven dried to completely remove moisture. The weight of each biomass after drying was obtained using a digital weighing balance. Thereafter the samples were milled and sieved with a 150 µm sieve to obtain uniform particle size.

3.2. Characterization of Biomasses

5 g of each sample was weighed and put into 2 different crucibles of known weight. Their weights were measured with the aid of the weighing balance. The crucibles were put in the oven at 105 ^{o}C for 3 hours at an interval of 30 minutes. After oven drying, the samples were kept in the desiccator for 15mins to cool before the weight of the samples were taken and recorded. In order to be sure that the samples were void of moisture, they were further dried in the oven for another hour at 105 ^{o}C and at same time interval until a constant weight was obtained on the weighing balance. The samples were then cooled in the desiccator for 15mins. The moisture contents of the samples were calculated using (1).

$$\% \text{ (w/w) Moisture Content} = \frac{Wa-Wb}{Wa}\ x\ 100 \quad (1)$$

$W_a = W_s - W_c$, $W_d = W_{fc} - W_d$, W_a = Weight of raw sample, Ws= Weight of raw sample + Weight of crucible.

W_c = Weight of crucible, W_{fc} = Weight of dry sample + Weight of crucible, W_b= Weight of dry sample ($w_{fc} - w_c$).

3.2.1. Determination of extractive contents in the biomasses

The above dried samples of constant weights were collected on filter papers with their dry weights noted. The water bath was switched on and its temperature was adjusted to 70 ^{o}C with a volumetric flask. 150 ml of acetone was measured and transferred into a round bottom flask. The Soxhlet extractor was set up, 2.5g of sample was measured and placed in the cellulose thimble, after which it was immersed in the water bath set at 70 ^{o}C for 4 h. After extraction, the sample was air dried for 1 h and put into the oven set at 105 ^{o}C until a constant weight was

achieved. The samples were weighed and the values were recorded. % Extractives was obtained from (2). Of primary concern in this analysis, is the removal of majority of the extractives without classifying them.

$$\% \text{ (w/w) Extractives} = \frac{WT - Wse}{Wa}\ x\ 100 \quad (2)$$

$W_b = W_x - W_y$, $W_T = W_z - W_y$, W_{se} = Weight of sample after extraction, W_y = Weight of filter paper, W_x = Weight of filter paper + Weight of Sample after extraction, W_z = Weight of dry sample + filter before extraction, W_a = Weight of dry sample.

3.2.2. Procedure for Determination of Lignin and Ash Contents

This experiment was done in order to know the amount of lignin and ash present in the samples. There are 2 types of lignin to be accounted for i.e. soluble and insoluble lignin.

i. Insoluble lignin:

300 mg each of the extracted sample was weighed and kept inside a test tube. 3 ml of 98% H_2SO_4 was added. The mixtures were shaken gently, to ensure complete mixing. The samples were kept at room temperature for 2 h at an interval of 30 mins with continuous shaking so as to allow for complete hydrolysis. The samples were transferred to separate Erlenmeyer flasks and 84 ml of distilled water was added. The samples were autoclaved for 1 h at 121 ^{o}C and cooled to room temperature. The samples were filtered and then the residue was dried at 105 ^{o}C in an oven for about 4 h till a constant weight was achieved. The residue was ashed in a muffle furnace at 575 ^{0}C for 6 h. The mass of insoluble residue was obtained from calculation using (3).

$$\% \text{ (w/w) Insoluble lignin} = \frac{W_{IL}}{W_{DW}} \times 100\% \quad (3)$$

W_{DW} = Oven dry weight sample = 0.3g, W_{IL}= Weight of insoluble residue = Wo – Wc, Wo = Weight of Sample + Weight of crucible, Wc = Weight of empty crucible

ii. Soluble lignin:

3 ml of the filtrate from (i) above was taken to the UV-Spectrometer to determine the absorbance of the sample. The Soluble lignin was also obtained from calculation. Equation 3 was used to obtain the ASL in the samples.

$$\% \text{ (W/W) Soluble Lignin} = \frac{uv\ absorbance\ x\ volume\ of\ filterate}{\varepsilon \times ODW\ sample} \times 100\% \quad (4)$$

$\boldsymbol{\varepsilon}$ = 30 (absorptivity of biomass at specified wavelength) l/gcm, ODW Sample = Oven-dried sample weight= 0.3g

Volume of filtrate = 0.087 L

To determine the ash content, the residue (Klason lignin + insoluble ash) in " i " above, was kept in the muffle furnace for 6 h at 575 ^{o}C. The percent ash was and % insoluble lignin were obtained from (5) through to (7).

$$\% \text{ (w/w) Ash} = \frac{W_K}{W_{DA}} \times 100\% \quad (5)$$

W_{DA} = Oven dry weight sample, Wk = Weight of Ash = Wf – We, Wc = Weight of empty crucible, Wf = Weight of crucible + ash (After burning insoluble residue in furnace)

$$\% \text{ (w/w) Insoluble Lignin} = \% \text{ Insoluble Residue} - \% \text{ Ash} \quad (6)$$

% (w/w) Total Lignin = % Insoluble Lignin + % Soluble lignin (7)

3.3. Alkali-Hydrogen Peroxide Pretreatment of the Samples

100 ml of 1 – 3 % H_2O_2 and distilled water mixture was made from the 30% lab grade hydrogen peroxide. 1.8 g NaOH pellets was added to the mixture in order to raise the pH to 11.5 so as to prevent the formation of furfural, enhance hemicellulose degradation of hemicellulose. 6 g of each dried sample of Napier grass and corn Stover was added to separate conical flasks containing hydrogen peroxide and distilled water. The soaked samples were kept for 3 days to allow for good contact times. After 3 days, each mixture was poured into two other flasks and heated on hotplate with magnetic stirrer at temperatures of 85 and 105 °C for 1-2 h. The wet weights of the residues were then recorded. 2 g each of the wet-pair of treated biomass was dried at 105°C for 4 h so as to estimate the total dry solid left in the pretreated sample.

3.3.1 Determination H_2O_2 Volume for Pretreatment

To obtain 1 - 3 % H_2O_2 in 100 ml of hydrogen peroxide and distilled water mixture from 30% (v/w) H_2O_2

$$CaVa = CbVb \quad (8)$$

Ca = 1% H_2O_2, Va = 100 ml, Cb = 30% H_2O_2, Vb =?

Vb = 3.33 ml

The same procedure was repeated for 2% and 3% H_2O_2. After the pretreatment, the samples were separated by vacuum filtration. Hollocellulose was seen as pale yellow precipitate for Corn stover and as pale green precipitate for Napier grass. The supernatant solutions were adjusted to a pH of 5.0 by addition of a 72% w/w dilute H_2SO_4 in order to solubilize the acid soluble lignin. The amounts of acid soluble lignin removed, and that present in the filtrate were analyzed with UV spectrometer. The amount of Klason lignin or acid insoluble lignin removed was obtained as discussed in [22].

3.3.2 Determination of cellulose/hemicellulose contents of the biomass substrates

Borrowing a leaf from the extraction of hemicellulose by Aravantinos-Zafiris and Oreopoulou [1], for 1 g of each sample treated with 72% w/w H_2SO_4, the ASL, AIL and ash contents were estimated. 0.5 M was made. Two water baths were filled with distilled water and set at a temperature of 100 ^oC. 1 g of each sample was kept inside separate Erlenmeyer flasks. With a volumetric flask, 150 ml of NaOH was poured into the flasks containing the samples. The Erlenmeyer flasks were placed in the water baths and allowed to boil for 3.5 h in order to bring them to the desired temperature. Vacuum filtration of samples was done with the aid of the buchner filter and 64 buchner flask. The samples were washed with distilled water until the solution gave a pH value of 7 i.e. Each sample was then dried in the oven to a constant weight and the hemicellulose content was obtained from (9).

$$\% \text{ (w/w) Hemicellulose} = \left(\frac{Wo-Wp}{Wo}\right) - AIL \times 100\% \quad (9)$$

Wo = 1g, Wp =Wa –Wc, Wa = Weight of sample + weight of crucible, Wc = Weight of crucible. However, the % cellulose = 100% - % (extractives + ASL + AIL + moisture + ash + hemicellulose) were calculated; see Figure 1.

3.4. Enzyme Hydrolysis of Treated Biomass

In order to further process, the alkali-peroxide pretreated biomasses, cellulase enzyme was prepared and added to the pretreated samples for hydrolysis. One gram each, of all four pretreated samples was added to 300 ml of distilled water in four different conical flasks, and the pH was adjusted to 4.8. The enzyme loadings were 5.0

FPU/g dry biomass of cellulase. The flasks were stirred at a temperature of 50 °C and autoclaved. The samples were inactivated by increasing the temperature to and maintaining it at 80°C for 15 minutes. Finally, the concentration of glucose and total reducing sugars (TRS) were determined using a kit based on the glucose oxidase reaction (reagent GOD PAD) and the dinitrosalicylic acid method (DNS), respectively. Cellulase activity (*Trichoderma reesei*, produced by Sigma Aldrich) was 64.1 FPU/ml and the experiment lasted for 96 h.

3.4.1 DNS reagent calibration

Standard glucose concentrations in the range of 0.2-1 mg/ml were prepared. 0.5 ml of each sample was put in separate testtubes. 1.5 ml of DNS reagent was added to the contents in the testtubes. The mixtures were shaken properly in a fume cupboard to prevent the formation/release of phenol vapour. The samples were boiled in a water bath for 15 mins. They were cooled for a few minutes and 8 ml of distilled water was added. The absorbance was taken with the spectrometer at 550 nm. The calibration curve was then obtained; see Figure 7.

3.4.2 Glucose analysis

Samples hydrolyzed for 72 and 96 h were taken for glucose analysis since the maximum yield is expected to be at both times. The RANDOX glucose kit contains a buffer with a pH of 7.0 and the enzyme glucose oxidase. The glucose concentration was determined after enzymatic hydrolysis and the hydrogen peroxide which was used in pretreating the samples reacted with phenol which is one of the constituents of the RANDOX buffer to form a red-violet quinoneimine dye as indicator. The enzymatic digestibilities of the pretreated Napier grass and corn stover samples were measured by removing 0.5 ml aliquot at 2, 24, 72 and 96 h. 0.5 ml of each sample was taken and placed in a water bath for 15minutes at 100 ^{o}C in order to denature the enzymes. The test tubes were withdrawn. 1.5 ml of the DNS reagent was added to each test-tube using the pipette and the mixture was shaken thoroughly. The test-tubes containing the mixtures were placed back into the water bath whose temperature was adjusted to 100 ^{o}C for another 15 minutes. 1ml of 40% Rochelle salt solution was added to the warm mixture in the test-tubes, after which they were cooled for 3 minutes and 6 ml of distilled water was added. The absorbance of each sample was obtained. Water was used as the blank reagent. The reducing sugar concentrations were calculated using the calibration graph of Figure 7.

$$\text{Glucose Concentration (C) (g/l)} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard\ solution} \quad (10)$$

3.4.3 Reducing Sugar yield

3 test tubes were labelled appropriately. Test tube A was for the standard glucose solution and the other two were for the Napier and corn Stover samples. 1 ml of the reagent was poured into each testtube. 10 µl each of glucose solution and test samples were poured into their appropriate testtubes. The mixtures were shaken properly and incubated for 5 minutes at 37 ^{o}C. They were then filtered with their absorbance measured at a wavelength of 550 nm. The sugar yields were estimated using (11).

Reducing Sugar Yield:

$$Y = S \times D \times \frac{V}{W} \quad (11)$$

Y = Reducing sugar yield from enzymatic hydrolysis (mg/g of dry biomass), S = Sugar concentration in diluted sample (mg equivalent glucose/ml), D = Dilution factor, V = Working Volume (20 ml), W = Weight of dry biomass (g). Absorbance of Sample = 0.145, S = Concentration of the reducing sugar from the calibration graph or by interpolation using values in Table 10 = 0.119 mg/ml, V = Working Volume = 20 ml, D = Dilution Factor = 5ml enzyme to 20 ml distilled water. W = 0.928g (dry weight of the biomss)

3.5. Delignification Kinetics of Corn Stover and Napier Grass

According to [25], lignin degradation occurs in the initial (rapid) phase, bulk (dominant) phase, and the final residual (slow) phase. Parameters for these phases are presented in (12):

$$\frac{L_K}{L_{ko}} = a_i \exp(-k_i \times t) + a_b \exp(-k_b \times t) + a_r \exp(-k_r \times t) \quad (12)$$

t is the reference time during which data was collected; L_K = mass of Klason lignin at time t; L_{ko} = initial mass of Klason lignin at t = 0; a_i = maximum fraction of lignin fragments released at the initial stage; a_b = maximum fraction of lignin fragments released in the bulk phase; a_r = maximum fraction of lignin fragments released at the residual stage; and k_i, k_b, k_r are the reaction rate constants for the initial, bulk, and residual delignification stages, respectively [24]. In the case of lignin removal during soda pulping of bagasse, the delignification lacks an initial phase. The alkaline delignification of bagasse proceeds with a fast and high rate of lignin removal which is not a characteristic of the bulk phase [10]. Also, under these conditions, this work assumes that delignification of the biomasses took place only in the bulk phase hence, the model presented in (12) reduces to (13).

$$lnk = -k_b \times t + a^b \, lnk_0 \quad (13)$$

4. Results and Discussions

According to literature, lignocellulosic biomass is composed of major structural components of Cellulose, hemicellulose, lignin, extractives, ash and moisture. The results obtained for both samples are as contained in Table 11 and Figure 1 gives an illustration of the compositions of the constituents of the biomasses.

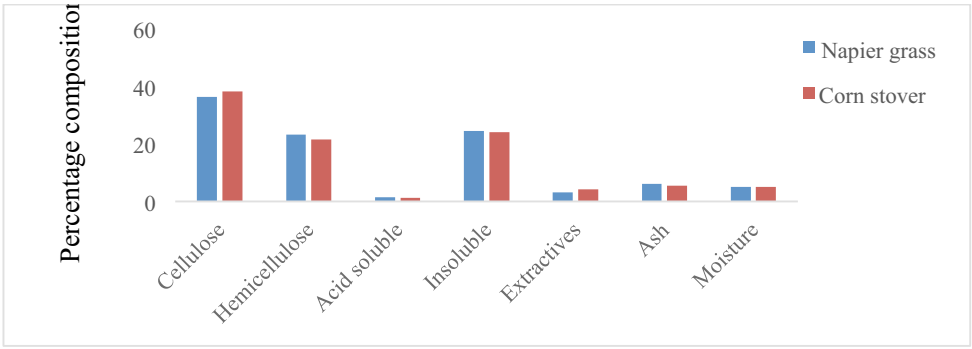


Figure 1: Percentage composition by weight (% w/w) of Napier Grass and Corn stover

From Figure 1, the ratio of cellulose to hemicellulose in the Napier grass sample (i.e. 36%: 21%) appears to be lower than that of corn stover whose cellulos: hemicellulose is 38%: 21%. Figures 2 and 3 give illustrations of the ASL removed between 0 and 60 minutes for both samples at 105 ^{0}C. Figures 4 and 5 show the amount of Klason (Acid insoluble lignin- AIL) lignin left in the biomasses after pretreatment at 85 ^{0}C and 105 ^{0}C. Since the amount of lignin removed after 60 and 120 mins at 85 and 105 ^{0}C were found to be lowest for 0.1 M pretreatment and highest for 0.3 M, the delignification kinetics of both pretreatment situations were then carried out in order to determine the kinetic parameters for the delignification processes. Kinetic data generated for the pretreated corn stover and Napier grass samples are as given in Table 1.

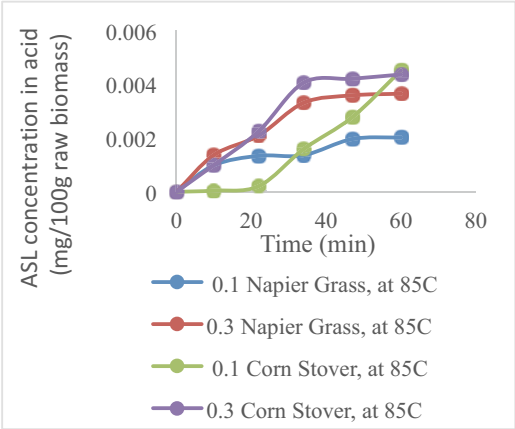


Figure 2: Variation of ASL with Time at 85 ^{0}C

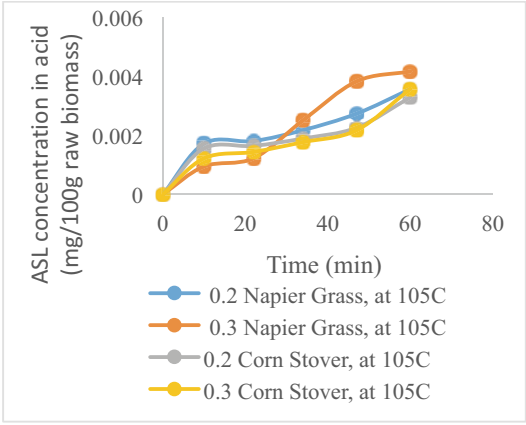


Figure 3: Variation of ASL with Time at 105 ^{0}

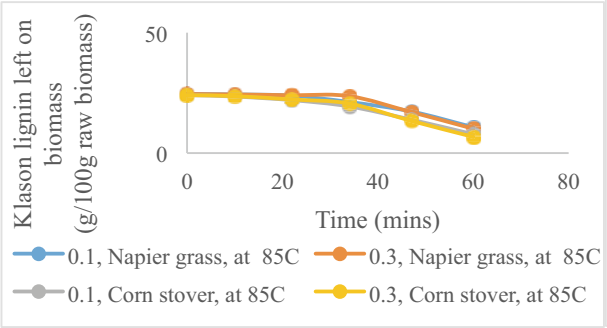


Figure 4: Lignin in Napier Grass and Corn Stover at 85 ^{0}C

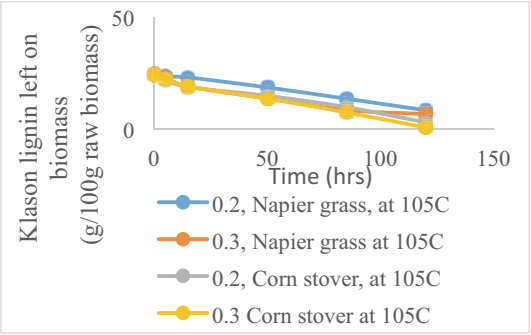


Figure 5: Lignin in Napier Grass and Corn Stover at 105 ^{0}C

Based on the data, graphs of lignin concentration versus time were obtained; Based on curve fitting tests conducted, the curves have good R^2 values. From the graphs, plots of ln R versus ln C_A were made; where R is the rate of lignin removal and C_A is the concentration of the lignin in solution. Using Differential Method of Analysis (DMA), the resulting polynomials were differentiated to obtain the rate. Equation 13 was then used to obtain the kinetic constants at 85 ^{0}C and 105 ^{0}C for both corn stover and Napier grass samples respectively (Figures 6a-h).

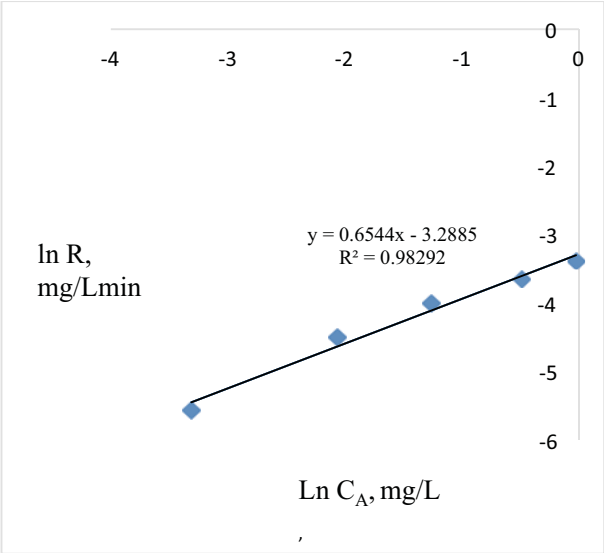
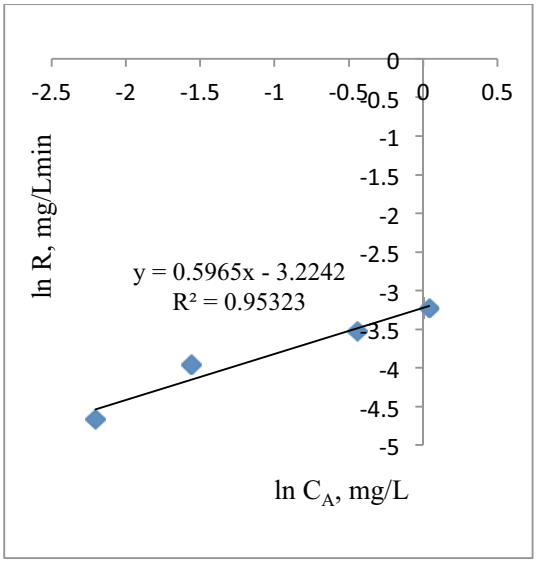
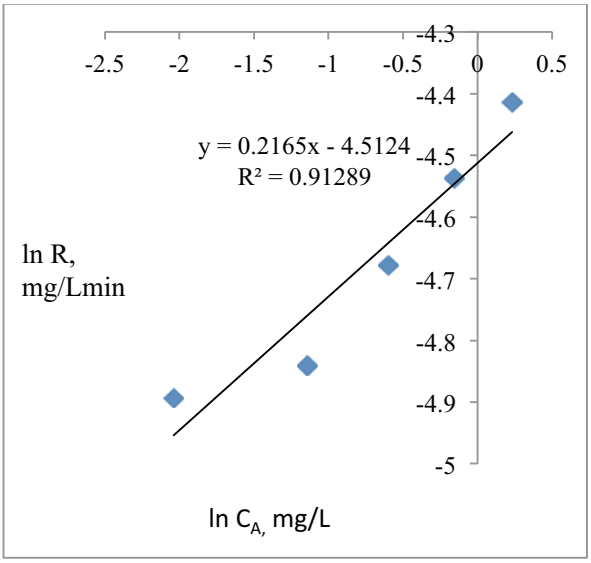


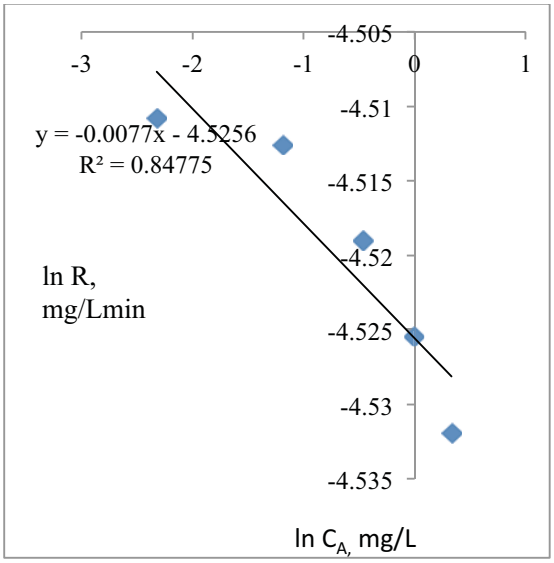
Figure 6a: ln R vs ln C_A for corn stover for 0.1M pretreatment at 85 ^{0}C



6b: ln R vs ln C_A for corn stover for 0.3M peroxide pretreatment at 85 ^{0}C



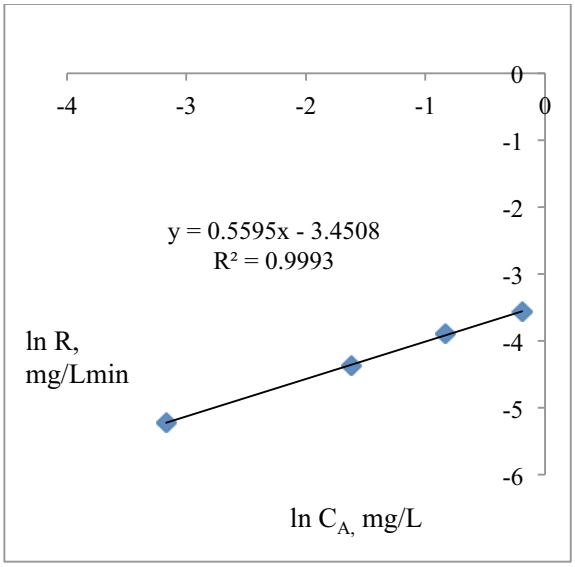
6c: ln R vs ln C_A for corn stover for 0.1 M peroxide pretreatment at 105 ^{0}C



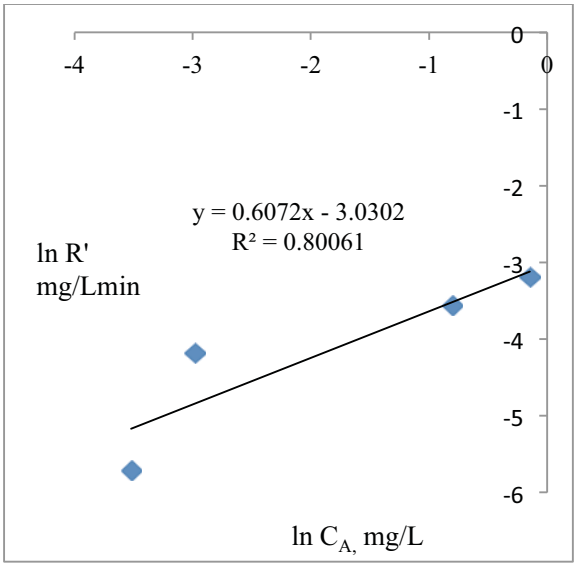
6d: ln R vs ln C_A for corn stover for 0.3M peroxide pretreatment at 105 ^{0}C

As shown in Table 1, k_1 is the rate constant for the reaction at 85^{0}C while k_2 is the rate constant for the reaction at 105^{0}C. The rate constants were seen to decrease at higher temperature which is justified by the Arhenius equation. The highest rate constant was obtained for 0.1 M hydrogen peroxide pretreatment of the grass. The rate constants are functions of the higher activation energies recorded at higher concentrations of the alkali. The concentration values obtained as well as the estimated rates of reaction were used to estimate the average rate constants in order to ensure accuracy. However, for 0.3 M peroxide pretreatment of Napier grass, the reaction

order was zero, hence, the delignification of Napier under such conditions is independent of the lignin concentration in the biomass.



6e: ln R vs ln C_A for Napier grass for 0.1M pretreatment at 85 ^{0}C



6f: ln R vs ln C_A for Napier grass for 0.3M peroxide peroxide pretreatment at 85 ^{0}C

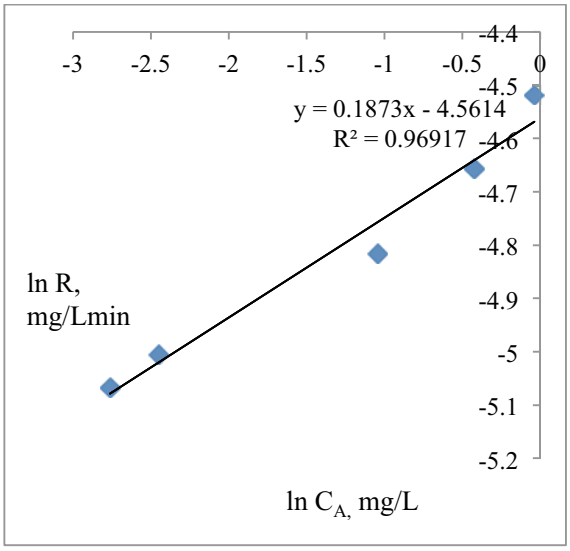


Figure 6g: ln R vs ln C_A for Napier grass for 0.1M peroxide pretreatment at 105 ^{0}C

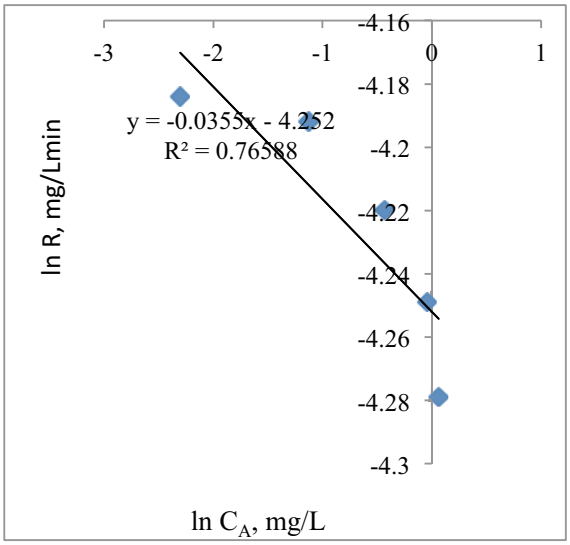


Figure 6h: ln R vs ln C_A for Napier grass for 0.3M peroxide pretreatment at 105 ^{0}C

From (13), the activation energies are 161.67 and 163.29 kJ/mol for 0.1M and 0.3M H_2O_2 pretreatments of corn stover respectively, and 64.76 and 71.66 kJ/mol for 0.1 M and 0.3M H_2O_2 pretreatments of Napier grass respectively. Based on the estimated activation energies, hydrogen peroxide pretreatment of Napier grass is preferred to corn stover for simple sugar production because, it is less energy intensive relative to corn stover; in this work, the results show that the effect of the peroxide in NaOH causes a change in the nature of the reactions of lignin in both substrates which implies that, based on the suggestions in [22] the reaction of lignin and hybrid

NaOH at higher concentrations i.e. > 0.1 M is altered by the presence of the peroxide thus making it endothermic.

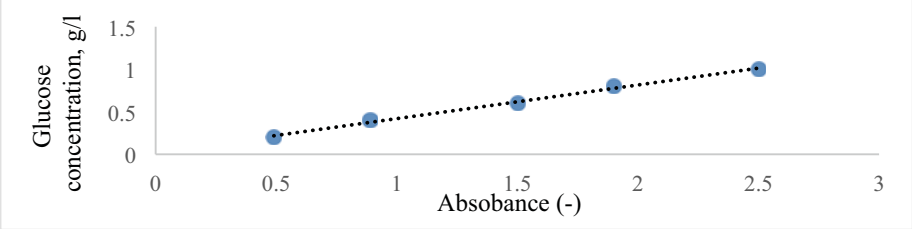


Figure 7: Glucose concentration vs absorbance

Table 1: Rate constant for the reactions at different Temperatures

Sample	**T ^{0}C**	**Rate constant**	**0.1M H_2O_2**	**0.3M H_2O_2**
Corn stover	85 ^{0}C	k_1	0.040mg$^{0.3}$/L$^{0.3}$min	0.040 mg$^{0.4}$/L$^{0.4}$min
	105 ^{0}C	k_2	0.0023 mg$^{0.8}$/L$^{0.8}$min	0.0022 mg/L min
Napier grass	85 ^{0}C	K_1	0.034 mg$^{0.4}$/L$^{0.4}$min	0.0520 mg$^{0.4}$/L$^{0.4}$min
	105 ^{0}C	K_2	0.011 mg$^{0.8}$/L$^{0.8}$min	0.015 mg/L. min

5. Conclusion

Peroxide pretreatment of Napier and corn stover increases access to their cellulose during enzyme hydrolysis. The activation energies obtained are 161.67 and 163.29 kJ/mol for 0.1M and 0.3 M H_2O_2 pretreatments respectively, for corn stover, while, for Napier grass, they are 64.76 and 71.66 kJ/mol for 0.1 M and 0.3 M H_2O_2 pretreatments respectively, hence, the reactions between lignin and the hybridized hydroxide-peroxide mix are endothermic for both substrates. Based results from the kinetic model, both delignification processes, raise an argument in favour of Napier grass for optimal production of simple sugars. The estimated reaction orders are in the range of 0 - 0.7.

Acknowledgment

The authors appreciate the management of Covenant University for their support all through this research.

References

[1] Aravin-Safaris, G and Oreopoulou, V. The Effect of Nitric Acid Extraction on Orange Pectin, *J. Sci. Food Agric*. 60:127-129 (1992).

[2] Granda, C.C.B. Sugarcane Juice Extraction and Preservation and Long-Term Lime Pretreatment of Bagasse, Ph.D Dissertation, Texas, A and M University (2004).

[3] Herbert, S. Handbook of Pulp and Paper. Wiley-VCH Verlag Gmb & Co, Weinheim, Germany (2006).

[4] Rabelo, S.C., Filho, R.M and Costa, A.C. A Comparison between Lime and Alkaline Hydrogen Peroxide Pretreatments of Sugarcane Bagasse for Ethanol Production, *Applied Biochemistry and Biotechnology*, 144(1): 87-100 (2008).

[5] Klemm, D., Heublein, B, Fink, H.P and Bohn, A. Cellulose: A Fascinating Biopolymer and Sustainable Raw Material. Angew Chem. Int. England, vol. 44, Series (Book 22), pp. 3358-3393 (2005).

[6] Nishiyama, Y., Langan, P and Chanzy, H. Crystal Structure and Hydrogen-Bonding System in Cellulose Iβ from Synchrotron X-Ray and Neutron Fiber Diffraction, *Journal of American Chemical Society,* 124(31): 9074-9082 (2002).

[7] Yang, T.C. Plant Fiber Chemistry, China Light Industry Press, Beijing, China, pp. 50-56 (2008).

[8] Mohammed, A.H. Conversion of Lignocellulosic Material into Fermentable Sugars. Ph.D Thesis, University of Berlin, pp. 1-49 (2012).

[9] Sjostron, E. (1993). Wood Chemistry Fundamentals and Applications, 2nd Edition, Academic Press, Orlando, USA, pp. 1-293 (1993).

[10] Katzen, R and Schell, D.J. Lignocellulosic Feed Stock Biorefinery: History and Plant Development for Biomass Hydrolysis. (*Eds*. Kamm, G, Grubber, P and Kamm, M) *In:* Biorefineries Industrial Processes and Products: Status Quo and Future Direction, Wiley-VCH, Weihein, 1: 129-138 (2006).

[11] Sarkanen, K.V and Hergert, H.L. Lignin: Occurrence, Formation, Structure and Reactions. (*Eds.* Sarkanen, K.V and Ludwig, C.H), Wiley Interscience, Chapter Four pp.95-99 (1971).

[12 Wood, T.M and Garcia-Campayo, V. Enzymology of Cellulose Degradation (*Eds.* Colin Ratledge) *In:* Physiology of Biodegradable Microorganisms. *Biodegradation,* 1: 147-161 (1990).

[13] Teymouri, F., Laureano-Perez, E., Alizadeh, H and Dale, B.E. Ammonia Fiber Explosion Treatment of Corn stover, *Applied Biochemistry and Biotechnology*, pp. 113-116: 951-963 (2004).

[14] Sun, Y and Cheng, J. Hydrolysis of Lignocellulosic Materials for Ethanol Production: A Review. *Bioresource Technology,* 83(1): 1-11 (2002).

[15] Bensah, E.C and Moses, M. Chemical Pretreatment Methods for the Production of Cellulosic Ethanol: Review Article. *International Journal of Chemical Engineering-Hindawi,* pp. 1-21 (2013).

[16] Gottschalk, G. Bacterial Metabolism. 2nd Edition, Springer-Verlag, New York, pp. 1-359 (1986).

[17] Carpeda, S.C. Introduction to Biomass and Energy Conversions, CRC Press, Taylor and Francis Group, pp. 1-645 (2013).

[18] Zhang, S. Revealing White Matter Fiber Structure with Diffusion Imaging. Ph.D Thesis, pp. 1-137 (2006).

[19] Chang, V. Burr, B and Holtzapple, M. Lime Pretreatment of Switch Grass. *Applied Biochemistry and Biotechnology,* 1: 63-65 (1997).

[20] Yang, T.C., Chou, C.C and Li, C.F. (2002). Preparation, Water Solubility and Rheological properties of the N-Alkylated Mono or Disaccharide Chitosan Derivatives, *Food Res. Int.* 35: 707-713.

[21] Sanni, S.E., Akinrinola, O., Yusuf, O.E., Fagbiele, O., Agboola, O. Chemical Kinetics of Alkaline Pretreatment of Napier Grass Prior Enzymatic Hydrolysis, Open Chemical Engineering Journal, 12: 36-56 (2018).

[21] Chang, V and Holtzapple, M. Fundamental Factors Affecting Biomass Enzymatic Reactivity, *Applied Biochemistry and Biotechnology*. 1: 84-86 (2000).

[22] Cardon, C.A., Sanchez, O.J and Gutierrez, L.F. Process Synthesis for Fuel Ethanol Production, Series (Book 32), pp. 1-415 (2009).

[23] Drapcho, C.M., Nhuan, P.N and Walker, T. Biofuels Engineering Process Technology, 1st Edition, Mc-Graw Hill, pp.1-371 (2008).

[24] Mosier et al. Features of Promising Technologies for Pretreatment, *Bioresource Technology,* 96(2005): 673-683 (2005).

[25] Drapcho, C.M., Nhuan, P.N and Walker, T. Biofuels Engineering Process Technology, 1st Edition, Mc-Graw Hill, pp.1-371 (2008).