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EFFECT OF PRETREATMENT METHOD ON THE HYDROLYSIS OF CORN COB AND SAWDUST

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ABSTRACT

Efficient cellulose hydrolysis remains one of the most challenging problems in attempting to convert cellulose wastes into fuels or chemicals, and pre-treatment has been found to be crucial step before enzymatic hydrolysis of these polymers can effectively take place. As a result various pre-treatment methods have been developed to facilitate these bio-conversion processes, and this research focuses on the effect of two pre-treatment methods such as low pressure steam and sulphuric acid pre-treatment to remove some of the components like lignin and hemicellulose which form structural barrier to enzymatic accessibility of cellulose in corn cobs and sawdust. The cellulosic materials were first dried in oven at 65^{o}C for 24 hours, and using solid to liquid ratio of 1:10, the two methods were carried out at resident times ranging from 10 - 40 minutes. The low pressure steam method involved heating the cellulosic materials in an autoclave at 120^{o}C and 1atm above the normal atmospheric pressure; for the second method, the dried cellulosic materials were refluxed in 5 % sulphuric acid at a temperature of 120^{o}C. Pre-treated samples were filtered and liquid fractions were analysed for the presence of reducing sugars, while solid residues were dried in the oven and weighed to measure the mass lost during pre-treatment as a pointer to lignin breakdown. It was observed that the mass lost increased with time for both pre-treatment methods, but the low pressure steam pre-treatment gave higher lignin and hemicellulose removal when compared to the sulphuric acid pre-treatment. The liquid fractions after pre-treatment were found to contain some reducing sugar which increased with pre-treatment time and was higher in the corn prehydrolysate. The pre-treated materials were hydrolysed with two combinations of commercial enzymes namely cellulase/ hemicellulase and cellulase/β glucosidase. The reducing sugar was measured using Dinitrosalycilic acid (DNSA) method and the sugar yields from corn cobs were higher than that of sawdust when subjected to similar process conditions, and the enzyme combination of cellulase/glucosidase gave higher yields of reducing sugars. A modified form of the model equation used to describe the basic hydrolysis process gave a good fit the experimental data obtained.

Keywords: Pre-treatment, Sawdust, Corn cob, Hydrolysis, Sugar, Model

1. INTRODUCTION

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$. It is a polysaccharide consisting of a linear chain of several hundred to over ten thousand β (1→4) linked D-glucose units. Like starch, cellulose is a polymer which consists of glucose monomer units, but the glucose monomers units are linked together by β (1→4) linkage as opposed to α (1→4) linkage in insoluble starch [1].

Cellulosic materials are the most abundant and renewable biopolymer and they dominate waste material from agriculture and manufacturing/processing industries. The main compositions of these waste materials however, are cellulose, hemicellulose and lignin [2 - 4]. In other words, the cellulose component in cellulosic waste materials does not exist in isolation but usually co-exist, mainly with hemicellulose and lignin. The lignin is closely bound to the cellulose and hemicellulose and function to provide rigidity and cohesion to the material cell wall. This poses a structural barrier which limits enzyme accessibility to the cellulose during enzymatic cellulose conversion processes [5].

Pre-treatment is a necessary step to alter the structure of the cellulosic biomass in order to increase the rate and yield of enzymatic cellulose bio-conversion in cellulosic materials and to this effect, several pre-treatment methods have been developed. Generally, an ideal pre-treatment stage is to enable a

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high conversion of the cellulose and hemicellulose to simple sugars. [2]. The choice of a pre-treatment method, to a large extent still depends on the cellulosic waste materials and proposed utilization of the cellulosic waste fractions that will be obtained after pre-treatment [6].

The objective of this study is to obtain pre-treatment conditions that will give high hemicellulose and lignin removal, high cellulose conversion to fermentable sugars and a model equation which could describe the hydrolysis of the pre-treated samples. To this effect, corn cob and sawdust were subjected to low pressure steam and 5% sulphuric acid pre-treatments with the expectation that the various structural components of these materials will behave differently in both situations. It has been suggested that under high temperature and in acidic condition, lignin will agglomerate into smaller particles, separate from cellulose and then undergo condensation reactions that prevent its dissolution which may subsequently result in removal of lignin in small amounts. It is also expected that the amorphous nature of hemicellulose unlike the crystalline nature of cellulose will make it to hydrolyse much more rapidly under high temperature and in dilute acid media [7].

2. EXPERIMENTAL

2.1. Materials

Corncobs, sawdust, de-ionized water, sulphuric acid (H_2SO_4), cotton wool, aluminium foil, filter paper, pH meter, 100ml capped bottles, funnel, retort stand, weighing scale, mesh 40, mesh 80 screens, glass beaker, standard vacuum flask, Buchner filter funnel, measuring cylinder, milling machine, autoclave machine, reflux apparatus; 2-necked round-bottomed flask, vacuum pump, reflux column, heating mantle, condenser, shaker incubator. The experiments were carried out in triplicates

2.2. Preparation of Samples

Corn cobs were obtained from farmers, dried in an oven at 65^{o}C for 24hr and milled in a milling machine while sawdust was collected from saw mill and also dried. Both materials were then separately screened and particles which passed through 40 mesh (400μm) screens but retained on 80 mesh (177 μm) screens were taken as experimental samples.

2.3. Acid Pre-treatment of Samples

30g of samples of both corncobs and sawdust were each weighed and placed into several 500ml beakers and labelled appropriately. 300ml of 5% sulphuric acid was weighed and emptied into each beaker thus ensuring the solid-liquid ratio 1:10. Taking one beaker at a time, the content of each beaker was poured into the round bottom flask of the reflux apparatus and the heating mantle was set to 120^{o}C and refluxes were carried out for 10, 20, 30 and 40 minutes. After the elapse of pre-treatment time, the contents in the round bottom flask were removed and pre-hydrolysates filtered to separate liquid and solid phase by using a vacuum pump. The liquid factions collected during the filtration processes were analyzed for dissolved reducing sugar using glucose as standard, while the solid residues were thoroughly washed several times with tap water to remove the acid. These solid residues were dried in the oven at 65^{o}C for 24hours and then weighed to determine the mass lost.

2.4. Low Pressure Steam (LPS) Pre-treatment of Samples

Similarly, as was done for the acid pre-treatment method, 30 g of samples of each material were placed in several 500 ml beakers and 300 ml of water was added to each beaker to obtain a solid-liquid ratio of 1:10. The beakers were then covered with aluminium foil and placed inside a closed and pressurized vessel (autoclave).The autoclave which served as the reactor was heated to a temperature of about 120 ºC at a pressure of 1 atmosphere above normal atmospheric pressure for

periods of time ranging from 10 to 40 min [8-10]. After each treatment, the liquid phase was separated from the solids by filtration with the aid of vacuum pump. The liquid factions collected during the filtration process were also analysed for sugar while the solid residues were thoroughly washed, dried in the oven at 65ºC for 24 hours and weighed to obtain the mass lost.

2.5. Hydrolysis of the Solid Residue with Enzymes

Hydrolysis was carried out on the corn cob and sawdust pre-treated for 20 to 40 minutes in a shaker incubator at temperature of 45 ºC, pH 5.0 and rotation speed of 120 rpm. Three commercial enzymes; cellulase with an activity of 1000BHU/g, hemicellulase with an activity of 45FBG/g and β-glucosidase with an activity of 250CBU/g, obtained from Novozymes were used for the hydrolysis process. A set of the pre-treated samples were hydrolysed using a combination of cellulase and hemicellulase while another set were hydrolysed using combination of cellulase and β-glucosidase. Based on Novozymes recommendations, the enzyme loading were 2g enzyme complex/100 g biomass, 0.4g enzyme complex/100 g biomass, 0.4g enzyme complex/100 g biomass for the cellulase, hemicellulase and β-glucosidase respectively. Untreated samples hydrolysed using the same sets of enzyme combination served as the control for the entire process. The solid/liquid ratio was 5% (w/v). Hydrolysis was monitored for 60 hours with samples withdrawn for analysis at intervals of 12 hours.

2.6. Analytical Methods

The compositional analysis of biomass was done as previously described by Muhammad et al., [11]. Soxhlet extractor was used to determine the amount of extractives; 120 mL of acetone was used as solvent on 2g of dried biomass for extraction at 90°C for 2h. The residues that remained after the removal of the extraction solvents were dried at 105°C. The amounts of the extractives were then calculated from the weight differences before and after the extraction [12, 13]. For the determination of hemicellulose content, 2g of dried, extractive free biomass samples were heated at 80°C for 2h in 20.0 mL of 0.5 mol/dm^3 solution of sodium hydroxide; the biomass recovered from this treatment were washed with deionized water until a neutral pH is reached and dried to a constant weight. The differences in biomass weight before and after the treatment gave the hemicellulose [12, 13]. Lignin contents were determined by adding 30.0 mL of 98% sulphuric acid to 1g dry extractive-free biomass samples, they were left for 24h at ambient temperature, and then boiled at 100°C for 1h. The mixtures were filtered, and the residues were washed with deionized water until complete removal of the sulphate ion. The residues were then dried to a constant weight to give the weight of the lignin [12, 13]. The cellulose contents were calculated by difference, assuming that hemicellulose, lignin and extractives are the only components of the entire biomass [12-14].

Reducing sugars in the liquid fraction from pre-treatments and hydrolysis were estimated with DNSA [15] using glucose as standard.

2.7. Data Analysis

Estimations of the concentration of reducing sugar (RS) by DNSA method is given in g/L
The percentage cellulose conversion

$$= \frac{concentration\ of\ RS\ in\ hydrolysate[{}^{g}/_{L}]}{1.1 \times substrate[{}^{g}/_{L}]} \times 100\%$$

Where substrate $\left[{}^{g}/_{L}\right]$ = fraction of cellulose $\left[{}^{g}/_{g}\right]$ in the dry biomass used for the hydrolysis multiplied by the quantity of biomass $\left[{}^{g}/_{L}\right]$ and

Quantity of biomass $\left[{}^{g}/_{L}\right] = \frac{quantity\ of\ dry\ biomass\ used\ for\ hydrolysis[g]}{Total\ hydrolysis\ volume[ml]} \times \frac{1000ml}{L}$

2.8. Modification of Model Equation

A modified form of the model equation that was derived by Zhang et al., [16] was used to fit the experimental data obtained for the hydrolysis. Derivation of the model is based on the fact that the process of enzymatic hydrolysis of cellulose to reducing sugar involves the formation of an intermediate substrate-enzyme complex before the final formation of the sugar; this can be represented by the equation below:

$$[E] + [S] \underset{k_1'}{\overset{k_1}{\leftrightarrow}} [ES] \underset{k_2'}{\overset{k_2}{\leftrightarrow}} [E] + [P] \quad (1)$$

Considering the series consecutive reversible reactions above, for the formation of ES, $K_{C1} = k_1/k_1'$. Also, for the decomposition of ES to form the Enzyme and product, $K_{C2} = k_2/k_2'$
Where:

K_{C1} = equilibrium constant for the reaction involving the formation of enzyme-substrate complex

K_{C2} = equilibrium constant for the reaction involving the formation of enzyme and substrate

E = Enzyme concentration, S = Substrate (cellulose) concentration, P = Product (reducing sugar) concentration, k_1, k_2, k_1', k_2' are the reaction rate constants, S_o = Initial substrate (cellulose) concentration, ES = Substrate-Enzyme complex concentration.

The rate equation with respect to the substrate complex is given by:

$$\frac{d[ES]}{dt} = k_1[E][S] + k_2'[E][P] - k_1'[ES] - k_2[ES] \quad (2)$$

The rate equation with respect to the product (reducing sugar) is:

$$\frac{d[P]}{dt} = k_2[ES] - k_2'[E][P] \quad (3)$$

Assuming the rate of de-polymerization of the substrate (cellulose) is far greater than the rate of re-polymerization, then:

$$k_2 \gg k_2'$$

Equation (2) becomes:

$$\frac{d[ES]}{dt} = k_1[E][S] - k_1'[ES] - k_2[ES] \quad (4)$$

While equation (3) becomes:

$$\frac{d[P]}{dt} = k_2[ES] \quad (5)$$

Assuming quasi equilibrium, equation (4) becomes

$0 = k_1[E][S] - k_1^{'}[ES] - k_2[ES]$

$$k_2[ES] + k_1^{'}[ES] = k_1[E][S] \tag{6}$$

Carrying out material balance on the substrate,

$[S_o] = [S] + [P] + [ES]$

This implies,

$$[S] = [S_o] - [P] - [ES] \tag{7}$$

Putting (7) into (6);

$k_2[ES] + k_1^{'}[ES] = k_1[E]([S_o] - [P] - [ES])$

Expanding and collecting like terms;

$k_2[ES] + k_1^{'}[ES] = k_1[E][S_o] - k_1[E][P] - k_1[E][ES]$

$[ES]\left((k_2 + k_1^{'}) + k_1[E]\right) = k_1[E]([S_o] - [P])$

Making $[ES]$ explicit;

$$[ES] = \frac{k_1[E]([S_o] - [P])}{(k_2 + k_1^{'}) + k_1[E]} \tag{8}$$

Taking the inverse of equation (8)

$$\frac{1}{[ES]} = \frac{k_2 + k_1^{'}}{k_1[E]([S_o] - [P])} + \frac{k_1[E]}{k_1[E]([S_o] - [P])} \tag{9}$$

Equilibrium constant $K_e$is given by:

$$K_e = \frac{k_2 + k_1^{'}}{k_1} \tag{10}$$

Putting equation (10) into (9) then factoring and inverting

$$[ES] = \frac{[E]([S_o] - [P])}{K_e + [E]} \tag{11}$$

Putting equation (11) into equation (5) and separating the variables and integrating

$\int_0^P \frac{dP}{([S_o]-[P])} = k_2 \int_0^t \frac{[E]}{K_e+[E]} dt$

$-\ln\left[[S_o] - [P]\right]_0^P = k_2 \int_0^t \frac{[E]}{K_e+[E]} dt$

$[S_o] - [P] = [S_o] e^{-k_2 \int_0^t \frac{[E]}{K_e+[E]} dt}$

Collecting like terms to make P explicit;

$$P = [S_o]\left[1 - e^{-k_2 \int_0^t \frac{[E]}{K_e+[E]}dt}\right] \tag{12}$$

Assuming second order deactivation of enzyme,

$$\frac{d[E]}{dt} = -k_d[E]^2 \tag{13}$$

Separating variables and integrating from E_o to E, the equation (13) followed by factorization it gives

$$[E] = \frac{[E_o]}{1 + [E_o]k_d t} \tag{14}$$

Putting equation (14) into equation (12) gives equation (15)

$$[P] = [S_o]\left[1 - \left(1 + \frac{[E_o]k_d K_e t}{K_e + [E_o]}\right)^{-\frac{k_2}{k_d K_e}}\right] \tag{15}$$

Based on the chemistry of the reaction involving the addition of water to cellulose to yield glucose, Equation (15) was modified by multiplying its right side by 1.1 as conversion factor. Thus, modified form becomes:

$$[P] = 1.1 \times [S_o]\left[1 - \left(1 + \frac{[E_o]k_d K_e t}{K_e + [E_o]}\right)^{-\frac{k_2}{k_d K_e}}\right] \tag{16}$$

Computation of the percentage conversion of cellulose in each of the substrates were done using the following modified form of equation (16)

$$\frac{P}{1.1 \times S_o} \times 100\% = \left[1 - \left(1 + \frac{[E_o]k_d K_e t}{K_e + [E_o]}\right)^{-\frac{k_2}{k_d K_e}}\right] \times 100\% \tag{17}$$

2.9. Estimation of Model Parameters and Simulation of Hydrolysis Data

In order to fit the experimental data and estimate the values of the constants in the model equation, least squares method using Levenberg-Marquardt algorithm for curve fitting in Matlab 2012 environment was used. Experimental data were grouped based on the following

1. Initial enzyme concentration, E_o used for the hydrolysis was constant and has a value of 1.20g/l
2. The type of enzyme used had effect on the rate of hydrolysis
3. The hydrolysis reaction is substrate specific

The third and fourth assumptions led to the division of the experimental data into four different categories: corn cob hydrolysed using combination of cellulase and hemicellulase, sawdust hydrolysed using combination of cellulase and hemicellulase, corn cob hydrolysed using combination of cellulase and β-glucosidase, and sawdust hydrolysed using combination of cellulase and β-glucosidase. The results obtained are assumed valid under these conditions.

3. RESULTS AND DISCUSSION

3.1. Effect of Pre-treatment on the Loss of Mass

It is important to know the weight of the solid mass residue after pre-treatment of cellulosic material because it is an indicator of the type/composition of cellulosic material and the effect of the pre-treatment method. Figure 1 shows the trend of mass loss when corn cob and sawdust were subjected to 5% sulphuric acid and LPS pre-treatments at various time intervals.

It could be observed from the results obtained that there was a decline in the solid mass recovered with increase in pre-treatment time, and hence an increased loss of weight as a function of pre-treatment times. In other words, a higher pre-treatment time provided a higher removal of components. The loss in mass may be due to hemicellulose solubilisation and lignin removal during pre-treatment.

From Figure 1, the percentage loss of mass in the 5% sulphuric acid pre-treatment was lower than that of LPS pre-treatment; this may partly be due to the low residence pre-treatment times at which the experiments were conducted. Past researchers have shown that although dilute sulphuric acid at moderate temperature minimizes degradation of dissolved sugar from the removed hemicellulose, maximal lignin removal using low concentration (0.05%-5%) of sulphuric acid require longer period of treatment time [17]. In a comparative study conducted by Asma et al [17], the maximum weight loss (47.7%) of bagasse occurred on 4% sulphuric acid pre-treated for 180mins at 121°C, a result which proved to be better than the lignin removal and weight lost in bagasse treated with 10% sulphuric acid at 121°C for 15min [18], wheat straw treated with 1.5% sulphuric acid for 75 mins at 121ºC [19] and delignification of bagasse with 3.5 % Sodium Sulphite [20].

On the other hand, the ability of water to lower the softening point of lignin [21], depolymerize both lignin and hemicellulose [22, 23] may have resulted in higher mass loss in LPS pre-treatment relative to the dilute acid pre-treatment with doubtful ability to degrade lignin. Moreover, a number of volatile compounds from the degradation in LPS pre-treatment may be lost in steam in the advent of vapour loss. These may likely cumulate in higher mass loss observed in.

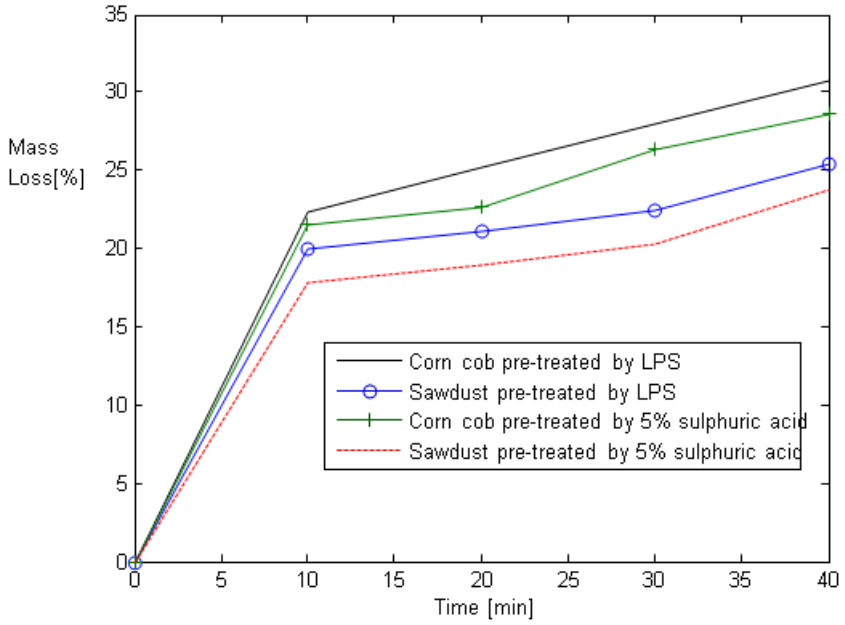

Figure 1. Loss of mass in pre-treated samples

3.2. Effect of Pre-Treatment on Component Removal

The results of the reducing sugar analyses of the prehydrolysates are shown in Figure 2 for the two cellulosic materials and the two pre-treatment methods. Previous researchers have observed that some level of hemicellulose solubilisation and removal occur during pre-treatments of cellulosic material, thus enhancing the accessibility of cellulose in the residual solids to enzymatic conversion [24]. The presence of sugar observed in the prehydrolysates has also been previously reported [25, 26].

From the results obtained as shown in Figure 2, it was observed that the possible solubilisation of the hemicellulose contents in the materials increased with increase in pre-treatment time in the sense that the amount of sugar observed in the prehydrolysates increased as the pre-treatment time increased. For corn cob pre-treated with 5 % Sulphuric acid, there was an increase in sugar yield from 19.04 to 24.70 g/l when the pre-treatment time was increased from 10 minutes to 40 minutes, while for corn cob pre-treated with low pressure steam, the increase in sugar yield was from 19.78 to 26.36 g/l. In the case of the sawdust pre-treated with 5 % Sulphuric acid, an increase from 10.69 to 12.95 g/l sugar yield was recorded while an increase from 11.74 to 14.21 g/l was recorded in sawdust pre-treated with low pressure steam. Taking the concentration of the reducing sugar as a pointer to hemicellulose solubilisation/removal from the biomasses, it can be inferred that pre-treatment using low pressure steam gave higher increase in hemicellulose solubilisation when compared with the 5% sulphuric acid pre-treatment. Furthermore, it can also be suggested that higher hemicellulose solubilisation was recorded in corn cob, relative to sawdust on the basis of the amount of sugar in the prehydrolysate.

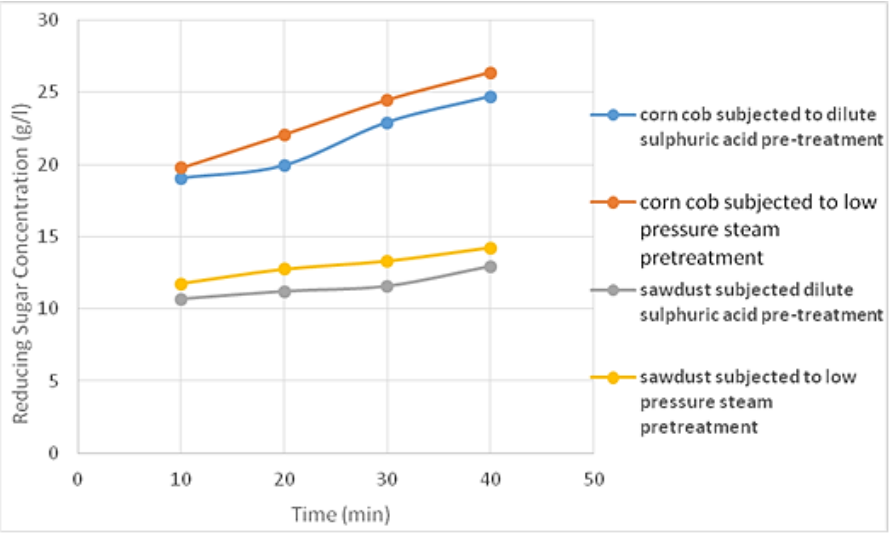

Figure 2. Effect of time on the RS present in prehydrolysate of both materials after 5% sulphuric and low pressure steam re-treatments

3.3. Chemical Composition of Biomass

The chemical composition of corn cobs and sawdust before and after subjecting them to 5 % sulphuric acid and low pressure steam pre-treatments for 10 to 40 minutes are shown Figures 3 and 4 respectively. Comparing the composition before and after pre-treatments of the materials, there were decreases in the percentages of hemicellulose and lignin, while the percentage composition of cellulose increased with increase in pre-treatment time. The percentage of hemicellulose reduction was greater than the percentage of lignin reduction, and this observation may be due to the differences in the structure of the three main components of the materials. The cellulose components of the materials are mostly crystalline in nature and so, its structure is not so easily broken at the pre-

treatment stage, especially when the pre-treatment condition is not harsh. The hemicellulose however is amorphous in nature and is easily degraded to yield some reducing sugars and in the process opening up the lignin-polysaccharides network which is dependent on severity of the pre-treatment and the rate of lignin depolymerisation. The lignin component in pre-treated sawdust still remained higher than that of pre-treated corn cob and this may imply higher barrier to the accessibility of the enzymes to cellulose in sawdust during hydrolysis.

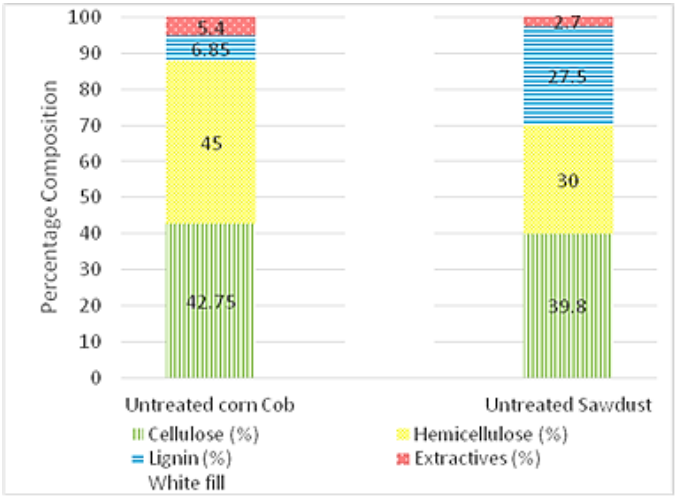

Figure 3. Chemical composition of untreated corn cob and sawdust

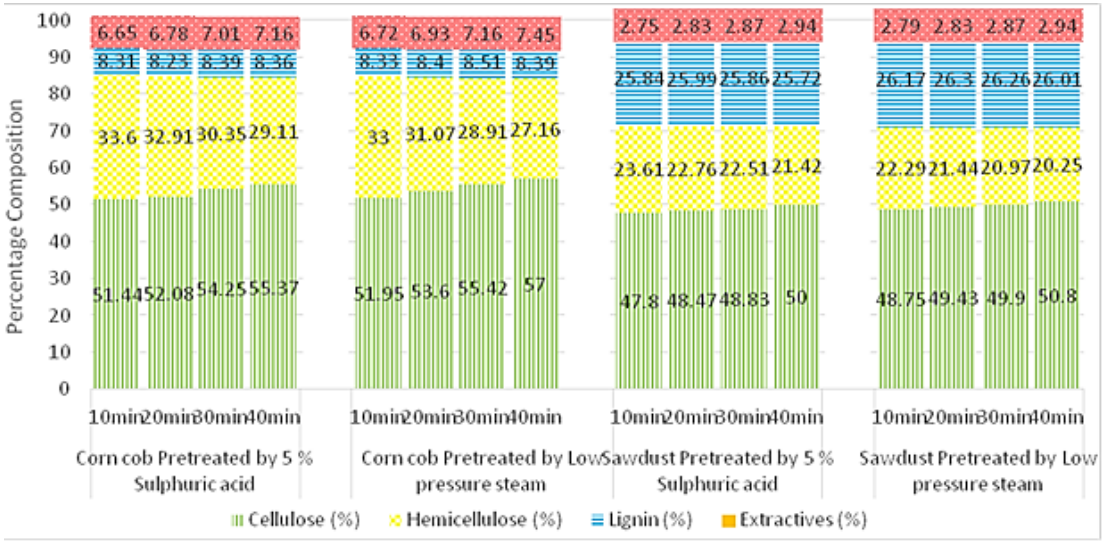

Figure 4. Chemical composition of pre-treated by corn cob and sawdust

Figure 5 shows the trends of lignin removal in the biomasses. Based on the untreated composition, pre-treated composition, initial and final masses of each the two materials it can be inferred that the estimated amount of mass loss due to lignin removal relative to the estimated amount of hemicellulose removed as sugar, from corn cobs and sawdust using the two pre-treatment methods was small; however, the highest amount of lignin removal of 0.29g/g lignin was recorded when sawdust was pre-treated using low pressure steam for 40 minutes. The rest of the results also showed that lignin removal during low pressure steam pre-treatment was higher than those of 5% sulphuric acid pre-treatment for similar experimental conditions.

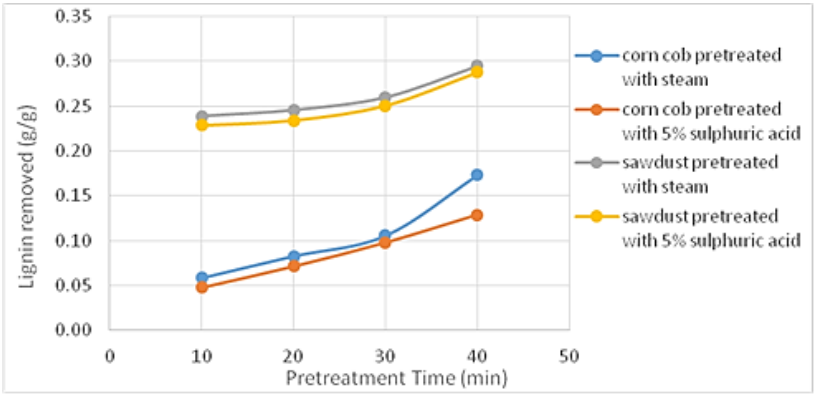

Figure 5. Estimation of lignin removed during pre-treatments

Although smaller amount of lignin removal was observed when compared to the hemicellulose that was solubilized, it is likely that the structure of lignin would have been affected by the removal of the hemicellulose thus increasing the prospect of interactions between cellulose and the enzymes in subsequent hydrolysis. The pre-treatment condition under which the highest hemicellulose solubilisation and lignin removal occurred was the low pressure steam method and at the longest pre-treatment time.

3.4. Enzymatic Hydrolysis of Corn cob and Sawdust

The results of the hydrolysis yield in terms of the percentage conversion of the cellulosic content of the pre-treated samples are shown in Table 1.

Table 1. Percentage conversion of cellulose in substrate to sugar during hydrolysis

Sample	Percentage cellulose conversion in substrate				
	12 hours	24 hours	36 hours	48 hours	60 hours
C12H	12.17	20.63	25.33	27.61	35
C14H	24.45	39.14	49.50	53.31	54.4
C22H	29.05	43.16	49.44	55.66	58.55
C24H	45.02	58.71	65.77	69.46	70.56
CupH	12.20	25.97	31.76	35.18	37.00
C12B	15.26	25.96	34.79	37.77	40.35
C14B	29.60	40.34	48.30	52.23	55.77
C22B	28.66	50.54	67.06	71.40	72.64
C24B	34.70	52.26	69.81	74.19	74.67
CupB	12.93	29.16	35.33	37.84	38.17
S12H	10.54	20.39	28.23	31.73	32.86
S14H	20.29	31.27	39.13	43.64	43.87
S22H	29.04	45.35	49.42	53.39	54.09
S24H	37.65	49.00	54.53	58.80	61.17
SupH	11.91	22.93	27.01	30.61	31.70
S12B	12.45	23.99	29.35	33.32	34.40
S14B	37.80	46.40	50.31	53.65	54.93
S22B	23.00	36.40	47.39	55.49	56.04
S24B	39.30	49.43	58.72	60.88	61.51
SupB	10.08	19.51	28.08	32.05	33.2

The results obtained indicated that pre-treatment proved to be a more effective approach for improving the cellulose conversion efficiency during enzymatic hydrolysis. Pre-treatment enhanced the reducing sugar yield in the materials by the removal of hemicellulose and lignin sheathing from

the lignocellulosic matrix, the low pressure steam pre-treatment however was found to be more suitable than the 5 % sulphuric acid pre-treatment on both the corn cob and sawdust used.

In all the cases examined, the rate of hydrolysis was high at the beginning of the process and reduced with time. The result also showed that the enzyme combination of cellulase with β-glucosidase gave higher maximum sugar yields most of the time. The maximum reducing sugar yield was obtained from corn cob pre-treated for 40 minutes by low pressure steam and hydrolysed using the combination of cellulase with β-glucosidase

In cases where samples were subjected to same pre-treatment condition and hydrolysed using the same enzyme combination, reducing sugar yield in corn cob was higher than that of sawdust. This observation may be due to the higher carbohydrate content in corn cob, a higher level of hemicellulose solubilisation in corn cob during pre-treatment and lesser lignin sheathing in corn cob when compared to sawdust.

3.5. Model Parameters

Figures 6 to 9 show the comparison between the experimental data and that obtained from the model equation. In virtually all the scenarios considered the model equation gave a good fit of the experimental data. The values of the constants as returned by the curve fitting processes are shown in Table 2.

When the constants obtained by the curve fitting process in Figure 6 are returned into the model, equation (16) becomes:

$$[P] = 1.1 \times [S_o]\left[1 - \left(1 + \frac{0.1259[E_o]t}{0.178 + [E_o]}\right)^{-0.315}\right] \tag{18}$$

When the constants obtained by the curve fitting process in Figure 7 are returned into the model, equation (16) becomes:

$$[P] = 1.1 \times [S_o]\left[1 - \left(1 + \frac{0.063[E_o]t}{0.09411 + [E_o]}\right)^{-0.5067}\right] \tag{19}$$

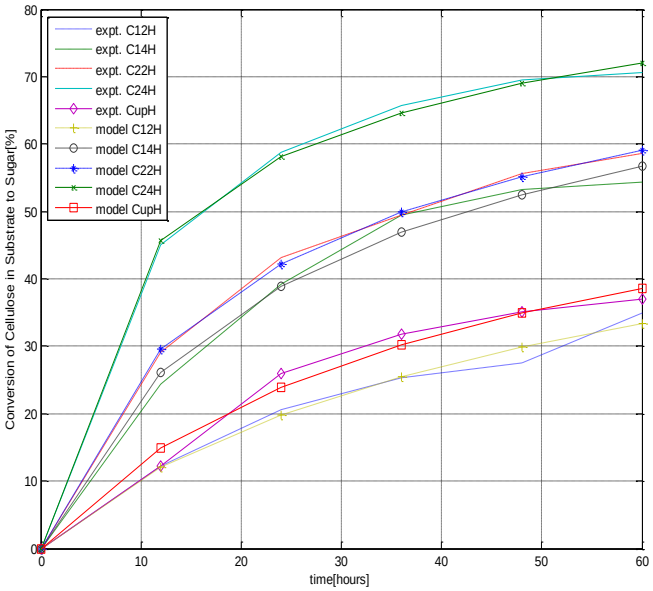

Figure 6. Comparison between experimental data and model output for corncob samples hydrolysed with mixture of cellulase and hemicellulase

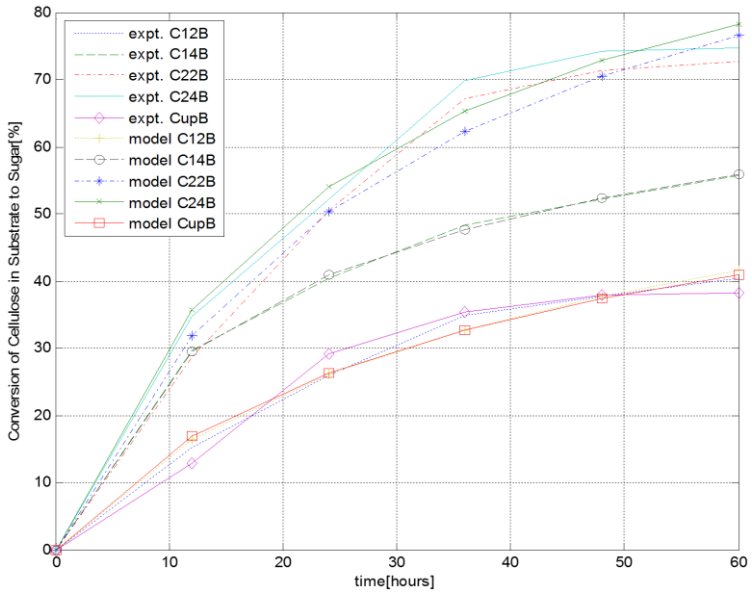

Figure 7. Comparison between experimental data and model output for corncob samples hydrolysed with mixture of cellulase and β-glucosidase

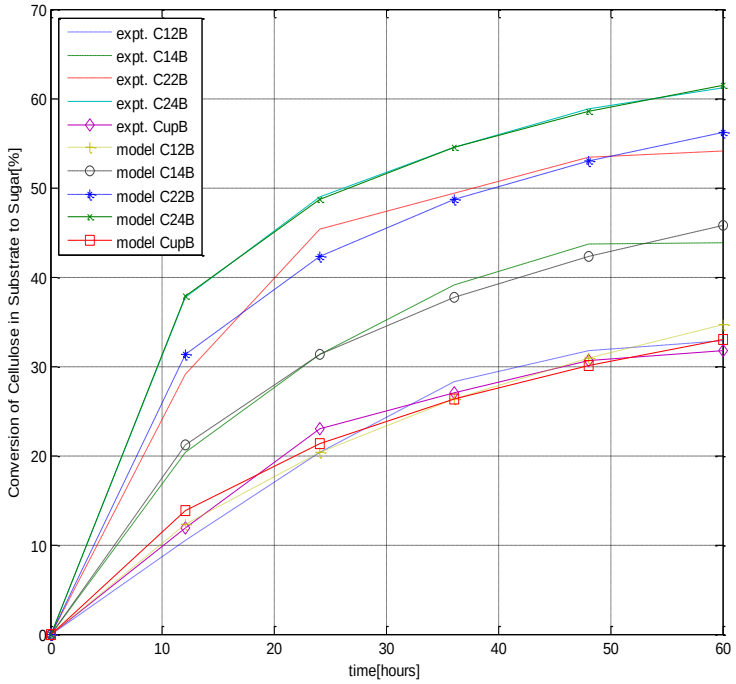

Figure 8. Comparison between experimental data and model output for sawdust samples hydrolysed with mixture of cellulase and hemicellulase

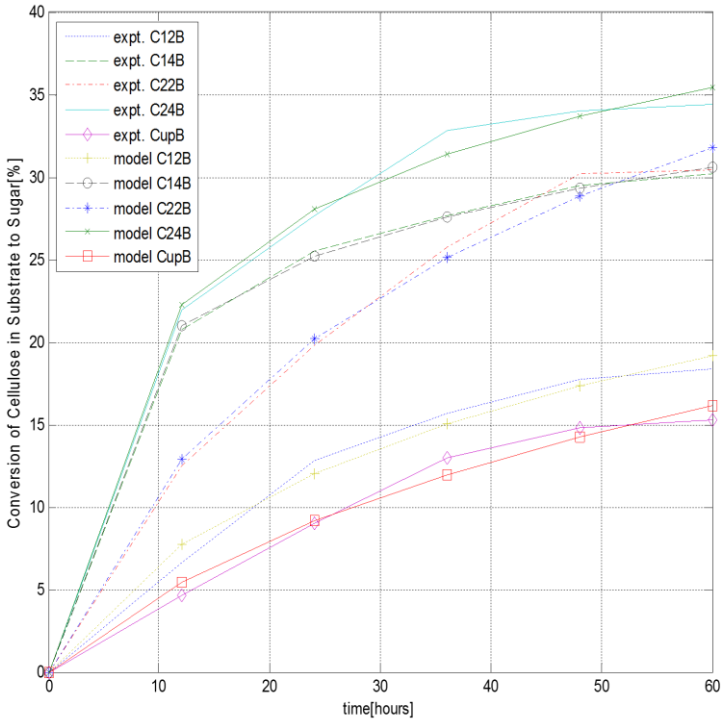

Figure 9. Comparison between experimental data and model output for sawdust samples hydrolysed with mixture of cellulase and β-glucosidase

$$[P] = 1.1 \times [S_o]\left[1 - \left(1 + \frac{0.1522[E_o]t}{0.2113 + [E_o]}\right)^{-0.2509}\right] \tag{20}$$

When the constants obtained by the curve fitting process in Figure 9 are returned into the model, equation (16) becomes:

$$[P] = 1.1 \times [S_o]\left[1 - \left(1 + \frac{0.260[E_o]t}{0.3366 + [E_o]}\right)^{-0.1073}\right] \tag{21}$$

Table 2. Constants returned by the curve fitting process

Case	Substrate	Model constants		
		K_e	k_d	k_2
1	Sawdust hydrolysed with cellulase and hemicellulase	0.2113	0.7205	0.0382
2	Sawdust hydrolysed with cellulase and β-glucosidase	0.3366	0.7712	0.0279
3	Corn cob hydrolysed with cellulase and hemicellulase	0.1780	0.7070	0.0397
4	Corn cob hydrolysed with cellulase and β-glucosidase	0.0941	0.6698	0.0319

Considering the series consecutive reversible reactions above, for the formation of ES, high K_{C1} (k_1/k_1') implies that k_1 is higher than k_1' thus, the formation of the enzyme substrate is favoured relative to the reverse reaction (i.e. the decomposition of ES to form the enzyme and substrate) and vice versa. Also, for the decomposition of ES to form the Enzyme and product, K_{C2} will be high for high k_2 relative to k_2'. This further implies that product formation is favoured by high value of k_2. However, we lumped all constants in order to obtain the overall equilibrium constant K_e, therefore, considering Table 2, a close look at the values obtained for different hydrolysed substrates (sawdust and corn cob) using different co-enzymes (hemicellulase and β-glucosidase; see cases 1& 4), higher

k_2 gave higher K_e and vice versa. The reason is because $k_{1\gg}k_1'$ for sawdust hydrolysed substrate is lower compared to that of corn cob based on Equation 10. Although for different substrates (sawdust and corn cob; cases 2 & 4), same co-enzyme (i.e. hemicellulase or β-glucosidase), higher k_2 gave lower K_e, thus implying that $k_{1\gg}k_1'$ for corn cob is higher relative to that for sawdust.

For same substrate (sawdust 1 & 2) hydrolysed with different co-enzymes, higher k_2 gave lower K_e and vice-versa. This implies that $k_{1\gg}k_1'$ is higher for hemicellulase hydrolysed sawdust relative to β-glucosidase hydrolysed sawdust whereas, for corn cob as substrate, higher k_2 gave higher K_e for hemicellulase hydrolysed corn cob relative to β-glucosidase hydrolysed corn cob, hence $k_{1\gg}k_1'$ is greater for β-glucosidase hydrolysed corn cob than that hydrolysed with hemicellulase. In addition, for both substrates and both co-enzymes, a higher deactivation constant, gave higher the equilibrium constant and vice-versa; see cases 3 & 4.

4. CONCLUSION

Pre-treatments proved to be an important process for maximizing the yield of enzymatic hydrolysis of corn cob and sawdust, and the effectiveness of both methods of pre-treatment was found to increase as the pre-treatment time increased.

Low pressure steam pre-treatment gave a higher lignin and hemicellulose removal when compared to the 5% sulphuric acid pre-treatment, and at the end of pre-treatment time of 40minutes, corn cob pre-treated using low pressure steam gave the highest reducing sugar yield.

Although the difference in maximum yields based on different combination of enzymes were marginal in some instances, the hydrolysis using combination of cellulase with β-glucosidase gave higher maximum sugar yields, and corn cob released higher reducing sugar under similar hydrolysis condition the sawdust.

The model equation yielded constants that are dependent on substrate types and enzyme combinations and gave good fits of the experimental data; based on the constants returned from the curve fitting, if obtaining a fast overall rate of reaction is of weightier importance in choice making, it may be preferable to hydrolyse sawdust with β-glucosidase as a co-enzyme while hydrolysing corn cob with hemicellulase as coenzyme with cellulase at these operating conditions.

Nomenclature

C12H is Corn cob samples pre-treated by 5% sulphuric acid for 20min and hydrolysed with combination of cellulase and hemicellulose

C14H is Corn cob samples pre-treated by 5% sulphuric acid for 40min and hydrolysed with combination of cellulase and hemicellulose

C22H is Corn cob samples pre-treated by LPS for 20min and hydrolysed with combination of cellulase and hemicellulose

C24H is Corn cob samples pre-treated by LPS for 40min and hydrolysed with combination of cellulase and hemicellulose

CupH is un-pretreated Corn cob Hydrolysed with combination of cellulase and hemicellulose

C12B is Corn cob samples pre-treated by 5% sulphuric acid for 20min and hydrolysed with combination of cellulase and β-glucosidase

C14B is Corn cob samples pre-treated by 5% sulphuric acid for 40min and hydrolysed with combination of cellulase and β-glucosidase

C22B is Corn cob samples pre-treated by LPS for 20min and hydrolysed with combination of cellulase and β-glucosidase

C24B is Corn cob samples pre-treated by LPS for 40min and hydrolysed with combination of cellulase and β-glucosidase

CupB is untreated Corn cob Hydrolysed with combination of cellulase and β-glucosidase

S12H is Sawdust samples pre-treated by sulphuric acid for 20min and hydrolyzed with combination of cellulase and hemicellulose

S14H is Sawdust samples pre-treated by sulphuric acid for 40min and hydrolysed with combination of cellulase and hemicellulose

S22H is Sawdust samples pre-treated by LPS for 20min and hydrolysed with combination of cellulase and hemicellulose

S24H is Sawdust samples pre-treated by LPS for 40min and hydrolysed with combination of cellulase and hemicellulose

SupH is untreated Sawdust Hydrolysed with combination of cellulase and hemicellulose

S12B is Sawdust samples pre-treated by 5% sulphuric acid for 20min and hydrolysed with combination of cellulase and β-glucosidase

S14B is Sawdust samples pre-treated by sulphuric acid for 40min and hydrolysed with combination of cellulase and β-glucosidase

S22B is Sawdust samples pre-treated by LPS for 20min and hydrolysed with combination of cellulase and β-glucosidase

S24B is Sawdust samples pre-treated by LPS for 40min and hydrolysed with combination of cellulase and β-glucosidase

SupB is untreated Sawdust Hydrolysed with combination of cellulase and β-glucosidase

BHU is biomass hydrolysis unit

CBU is cellobiase unit

FBG is fungal beta-glucanase unit

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