ALKALINE PEROXIDE OXIDATION PRETREATMENT OF CORN COB AND RICE HUSKS FOR BIOCONVERSION INTO BIO-COMMODITIES: PART A-ENZYMATIC CONVERTIBILITY OF PRETREATED RICE HUSKS TO REDUCING SUGAR

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ABSTRACT: In this study, a 2^3-central composite design (CCD) of experiments was adopted using MINITAB 15 statistical software to investigate the effect of pretreatment conditions on enzymatic digestibility of rice husks to reducing sugar and establish the optimum pretreatment conditions for the process. Alkaline peroxide oxidation pretreatment conditions were chosen at low–high values of 60–90 ^oC reaction temperature, 6–10 h reaction time, and 1–3%(v/v) H_2O_2 concentration. The optimized conditions were established and validated at 100 ^oC, 4.6 h, and 0.3% (v/v) H_2O_2 to obtain sugar yield of 246 mg/g after 96 h. Variations of enzyme and substrate loadings at the optimized pretreated conditions established that sugar yield increased to about 271 mg/g for 30 g/L biomass loading at 25 FPU/g biomass after 96 h and temperature of 45 ^oC. Increasing enzyme loading beyond 25 FPU/g biomass did not result in an increase in sugar yield. Stereoscope microscopy, scanning electron microscopy (SEM-EDX) images, and FTIR spectroscopy reveal physical and chemical changes to the rice husk after pretreatment and enzyme conversion. The pretreatment methodology investigated is suitable for lignocellulosic biomass such as rice husks to produce reasonable quantity of reducing sugar required to produce bio-commodities like biofuel and fine chemicals.
Keywords: Biomass, cellulose, lignocellulose, rice husks, enzymatic hydrolysis

1 INTRODUCTION

The gradual depletion of fossil fuels and the negative impacts such as greenhouse gas emissions into the atmosphere through combustion of these fuels has driven the world to utilize renewable-energy sources such as biofuel in order to reduce the total dependency on non-renewable energy sources. Industrialization of nations is growing speedily, and there is a progression in increasing demand for fuels attempting to satisfy both the industrial and domestic demands.

Second generation biofuel is based on raw materials rich in complex carbohydrates, resulting an interesting alternative to reduce competition with food industry. The process to obtain second generation biofuel such as ethanol involves four basic steps: feedstock pretreatment, enzymatic or acid hydrolysis, sugars fermentation, and ethanol recovery [1]. Lignocellulose is a generic term for describing the main constituents in most plants, namely cellulose, hemicelluloses, and lignin. It is a complex matrix, comprising many different polysaccharides, phenolic polymers and proteins. Lignocelluloses consists of a variety of materials with distinctive physical and chemical characteristics. It is the non-starch based fibrous part of plant material. Cellulose, the major component of cell walls of land plants, is a glucan polysaccharide containing large reservoirs of energy that provide real potential for conversion into biofuels. Cellulose is recalcitrant to biodegradation and needs to be hydrolysed in an initial pretreatment step into its constituent cellobiose units and into simpler D-glucose units in order to be liable to biochemical conversion. Rice husk represents 20% dry weight of harvested rice. It can serve as a low cost abundant feedstock for production of fuel [2]. They are considered waste materials because of their low value as animal feed due to low digestibility, peculiar size distribution, low bulk density, high ash/silica contents, and abrasive characteristics. However, rice husks also contain high quantities of ash and lignin, which combined with hemicelluloses results in a complex structure around the cellulose, being more difficult for its use as a lignocellulosic feedstock for conversion to ethanol.

In order to hydrolyze lignocellulosic biomass with enzymes successfully, it is important to apply a suitable pretreatment that can effectively disrupt linked lignin and crystalline cellulose. Pretreatment makes the polymers more accessible to the enzymes and thereby enhancing conversion into fermentable sugars [3]. Processes for bioconversion of lignocellulosic materials have been studied extensively. Examples include alkali [4], alkali-hydrogen peroxide/air [5],comminution, irradiation, steam explosion, hydrothermolysis, dilute acid, alkali, solvents [6], ammonia, SO_2, CO_2, and other chemicals [7].

In the present investigation, attempts were made to pretreat rice husk with the alkaline peroxide oxidation (APO) process such as to be amenable to enzymatic hydrolysis. Response surface methodology (RSM) based on central composite design (CCD) of experiments was adopted to investigate the optimum parameters of APO pretreatment such as to enhance enzymatic convertibility. The effects of three process parameters (reaction temperature, reaction time, and percent hydrogen peroxide concentration) on the pretreatment step were studied. The most suitable APO pretreatment conditions to obtain enriched solid fraction such that the enzymatic conversion process gave the maximum reducing sugar were selected and validated. Furthermore, at the optimized conditions, variations of enzyme and substrate loadings were evaluated. Stereoscope microscopy imaging and scanning electron microscopy (SEM) imaging were used to investigate changes brought about by the pretreatment and enzymatic hydrolysis of the raw biomass.

2 MATERIALS AND METHODS

2.1 Raw materials

Rice husk was sourced from a rice milling factory (Wasimi, South West, Nigeria; 6°59'N 3°13'E at 91 m elevation). The storage conditions of the raw material

before delivery to the laboratory was 26 °C to 32 °C, and after delivery the material was air-dried and kept in covered drums at ambient temperature, ranging from 23 °C to 25 °C. Samples were dried in a convection oven at 105 °C for 3 h to a dry matter content of 87%. The dried raw samples were further screened to get the required size fractions. Biomass samples ranged from 0.04 mm to 2.36 mm particle sizes. The screened sample fraction having sieve size of 1.18 mm was used for this study. All chemicals used in this study were of analytical grades. The raw material compositional analysis was carried out based on methods as reported earlier [5, 8]. Rice husk used in this study contained 25.05±0.47% cellulose and 25.66±1.39% hemicellulose. In addition, it contained 16.66±1.23% insoluble lignin and 0.92±0.29 soluble lignin (total lignin as 17.58±1.52), 26.98±0.58% ash, and 4.73±0.47% extractives.

2.2 Experimental design

A statistical 2^3 central composite design was used to develop a statistical model for the optimization of process variables. The CCD contains 20 experiments carried out in duplicate (Table I). The three variables chosen were designated as *A*(Temperature), *B*(Time), *C*(% v/v H_2O_2) each at five coded levels. The choice of these three factors were based on earlier reported studies on sawdust wood waste [9].

The model generated as a function of these variables on the predicted response of reducing sugar yield from pretreated biomass is a second-order polynomial and is represented as follows:

$$Y = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_{1,1} A + \alpha_{2,2} B^2 + \alpha_{3,3} C^2 + \alpha_{1,2} AB + \alpha_{1,3} AC + \alpha_{2,3} BC \quad \text{Equation...1}$$

where Y is the dependent variable, to are regression coefficients representing the linear, quadratic and cross-products of on the response; are the factors. The statistical software package MINITAB 15 (PA, USA) was used for regression analysis of experimental data, plotting of response surfaces in order to locate the optimum variables, and to optimize the process parameters. The coefficients in the second-order polynomial (Eq. (1)) were calculated by multiple regression analysis, based on the experimentally obtained data, and then the predicted responses for each experimental run were obtained using Eq. (1). All the experiments were performed in duplicate and the results presented are the average values. Analysis of variance (ANOVA) was used to test the significance and adequacy of the model.

2.3 Raw material pretreatment

Pre-treatment was conducted in 500 mL Erlenmeyer flasks. The flasks were loaded with 12.5 g dried rice husk. A slurry of the mixture was made by using solutions of hydrogen peroxide of different concentrations. Sodium hydroxide was added to the peroxide solutions to bring the pH of the medium to 11.5 (NaOH loading varied with percent hydrogen peroxide in mixture). Treatment of lignocelluloses with alkaline peroxide solution (pH 11.5–11.6) improves crop residues [10]. A mixture of slurry was made by adding water at a ratio of 20 g/g dry biomass. Percent H_2O_2 (volume by volume) in mixtures acted as oxidizing agent. Prior to pre-treatment in the air bath, the raw materials were soaked at room temperature (26 °C) for 72 h in the alkaline peroxide mixture. The flasks were placed in a temperature-controlled oven set at the corresponding reaction temperatures.

Table I: 2^3-level central composite design matrix, the experimental and predicted responses of reducing sugar yield

Run	Temp. (°C)	Time (h)	%H_2O_2 (v/v)	Reducing sugar (mg/g)	
				E[a]	P[b]
1	75	8	2	189.0	185.5
2	60	10	1	218.4	214.0
3	75	8	2	182.3	185.5
4	75	8	2	182.5	185.5
5	75	8	0.3	191.0	195.1
6	60	6	1	190.0	191.4
7	75	8	2	189.9	185.5
8	75	4.6	2	185.5	184.4
9	75	8	2	179.8	185.5
10	100.2	8	2	194.1	200.8
11	60	10	3	224.8	226.4
12	90	6	1	207.5	207.2
13	90	10	3	196.3	193.2
14	60	6	3	188.8	184.2
15	75	8	2	187.5	185.5
16	75	11.4	2	202.3	208.1
17	75	8	2	189.9	185.5
18	60	10	1	216.4	214.0
19	75	8	2	182.3	185.5
20	75	8	2	177.8	185.5
21	75	8	0.3	194.0	195.1
22	90	10	1	197.9	193.1
23	75	8	3.7	188.1	189.1
24	60	6	1	192.9	191.4
25	75	8	2	189.4	185.5
26	75	4,6	2	186.5	184.4
27	75	8	2	189.3	185.5
28	100.2	8	2	205.2	200.8
29	60	10	3	231.8	226.4
30	90	6	1	207.5	207.2
31	90	10	3	194.3	193.2
32	60	6	3	179.2	184.2
33	75	8	2	187.5	185.5
34	49.8	8	2	211.3	215.4
36	90	6	3	185.1	187.5
37	75	11.4	2	204.3	208.1

[a]E = Experimental values, [b]P = Predicted values.

Stirring was performed manually twice per day using stainless steel spatulas. Reaction was made to occur at the different temperatures, time, and hydrogen peroxide concentration (Table I). After the specified reaction time, the reaction vessels were allowed to cool to ambient temperature (26 °C). The pretreated materials were separated into the solid and liquid fractions by vacuum filtration, and the solid fraction was washed with water to a neutral pH. A portion of the wet solid sample was dried in the convention oven at 105 °C for 4 h to account for total solid remaining after pretreatment. The remaining part was stored frozen to be used later on for enzymatic digestibility.

2.4 Enzymatic hydrolysis

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/L dry biomass content). Sodium citrate buffer (5 mL, 0.1 M, pH 4.8), were added to the wet materials in 50 mL culture tubes. *Trichoderma reesei* cellulase enzyme system (EC 3.2.1.4) with an activity of 57.8 filter paper unit (FPU)/mL were added at a loading of 25 filter paper unit per g dry biomass (the dry biomass as the addition of cellulose and hemicellulose contents in treated materials). Distilled water was later added to bring total volume of mixture to 20 mL. Culture tubes were arranged in parallel and subsequently, sampling were carried out in parallel at intervals of 2, 24, 72, and 96 h hydrolysis periods (96 h period was used as the basis for optimization because of the production of highest fractions of sugar compared to other hydrolysis periods). The samples were boiled for 15 min in order to deactivate the enzyme. Samples were then centrifuged at 10,000 rpm for 10 min and the supernatant was used for sugar. Experiments were first conducted at 50 ^{o}C (later at the optimized condition, hydrolysis period was reduced to 45 ^{o}C) in a shaking incubator at 130 revolution minute [11, 12]. The amount of reducing sugars (RS) was calculated as milligram reducing sugar (as equivalent glucose) yield per gram of treated biomass.

Variations of enzymatic digestibility at the optimized conditions were evaluated at 3%, 4%, and 5% biomass loadings.

2.5 Morphological and elemental compositional analysis

The untreated, alkaline peroxide, and enzymatic treated biomass of rice husk samples were washed with distilled water and air dried for 72 h, and later stored in capped 50 mL-sized conical plastics for SEM-EDX analysis. The air dried samples were mounted on aluminium stubs using conductive carbon tape followed by sputter coating with carbon and gold-palladium at 5 nanometre scale. Biomass samples were examined using Carl-Zeiss Sigma scanning electron microscope (SEM) equipped with EDX mechanism, operated under vacuum between 7,23x10^{-10} –1.7x10^{-9} Torr for morphological and elemental analysis.

2.6 Stereomicroscopy

Air dried samples of the untreated, pretreated, and enzyme digested biomass were subjected to stereoscopic imaging. The samples were placed on a black background and images were captured using a Nikon SMZ745T stereomicroscope equipped with NIS-Element D Z-Series 7 software. Images were captured with a Nikon DS-Fi2 CCD camera operated by a Nikon Digital Sight System.

2.7 Fourier transform infrared spectroscopy

The untreated and pretreated enzyme digested samples were also evaluated for their surface chemistry with a PerkinElmer Frontier FT-IR spectrometer (PerkinElmer, USA) using the attenuated total reflectance (ATR) method. The crystal information of the samples was investigated. Six peaks related to the crystal system and degree of intermolecular regularity were taken into consideration, with three infrared ratios [13–15]. These ratios at different wavelengths have been used to measure relative cellulose crystallinities; α_{1437} cm^{-1}/α_{899} cm^{-1} (lateral order index (LOI)), α_{1378} cm^{-1}/α_{2900} cm^{-1}(total crystallinity index (TCI)), and α_{3400} cm^{-1}/α_{1320} cm^{-1} (hydrogen bond intensity (HBI)). The sample collection was obtained using 32 scans, in the range of 4000 to 400 cm^{-1}, at a resolution of 4 cm^{-1}.

3 RESULTS AND DISCUSSIONS

3.1 Enzymatic digestibility of APO pretreated rice husk

Reducing sugar yields as shown in Table I reveal that enhanced sugar production occurred at mild pretreatment temperatures and longer reaction periods (Pretreatment 29 having the highest sugar yield of 231.8 mg/g at 60 ^{o}C, 10 h, and 3%(v/v) H_2O_2 concentration). However, at these conditions more hydrogen peroxide concentrations are needed. This supports the fact that at very mild temperatures, chemical reactions proceed at a slower pace and also confirms the report that the concentrations of alkaline peroxide and temperature have compelling effects on cellulose convertibility as contained in other lignocellulosic feedstocks [16–18].

3.2 Statistical analysis of enzymatic digestibility

The experimental data as well as analysis of variance (ANOVA), the regression analysis and the plotting of response surfaces were performed to establish optimum conditions for the hydrolysis with MINITAB 15 statistical software and then interpreted. Application of CCD on the enzymatic process generated the following second order polynomial equations for reducing sugars yield as substituted from the model equation (Equ. 1);

$$Y = 277.312 + 2.757A + 6.362B - 15.264C + 0.035A^2 + 0.946B^2 + 2.336C^2 - 0.305AB - 0.207AC - 2.457BC \quad \text{Equation ...2}$$

Substituting the corresponding factors, A, B, and C as given in Table I into Equ. 2, the predicted responses were obtained. The predicted values were compared with the experimentally obtained values and the data were in close agreement (Table I and Fig. 1).

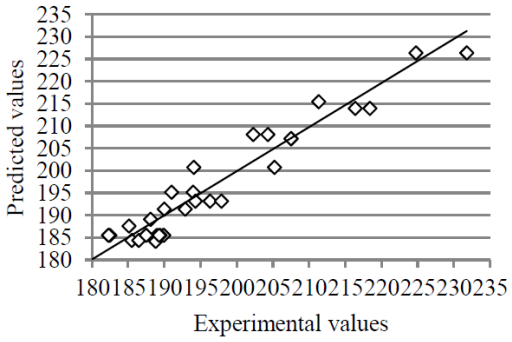


Figure 1: Experimental versus predicted reducing sugar yield values. The predicted reducing yield values were determined from the model equation

The regression analysis of the CCD and the ANOVA (analysis of variance) for surface response quadratic polynomial model of reducing sugars yield are given in Table II and Table III respectively. ANOVA was used to test the adequacy and fitness of the responses for linear, two function interactions and quadratic functions of the variables. A model with *p*-values ($p > f$) less than 0.05 was regarded as significant which corresponds to larger magnitude of *t*-value.

Table II: Results of regression analysis of the CCD for reducing sugar yield

Term	Coefficient	Standard error	*t*-value	*p*-value
constant	277.312	38.573	7.189	0.000
A	-2.757	0.709	-3.890	0.001
B	6.362	4.657	1.366	0.184
C	-15.264	8.436	-1.809	0.082
A^2	0.035	0.004	8.707	0.000
B^2	0.946	0.210	4.492	0.000
C^2	2.336	0.925	2.525	0.018
AB	-0.305	0.041	-7.505	0.000
AC	-0.207	0.081	-2.546	0.017
BC	2.457	0.601	4.091	0.000

R^2 = 91.47%; R^2 (Predicted) = 82.53%; R^2 (adjusted) = 88.52%

Table III: Analysis of variance for surface response quadratic polynomial model of reducing sugar yield

Source	Sum of square	Degree of freedom	Mean square	*f*-value	*p*-value
Model	5484.2	9	609.4	30.980	0.000
Linear	1741.2	3	163.3	8.300	0.000
Square	2003.7	3	634.9	32.280	0.000
Interaction	1739.3	3	579.8	29.480	0.000
Residual error	511.4	26	19.7		
Lack-of-fit	154	5	30.8	1.810	0.154
Pure error	357.4	21	17.0		

The lack-of-fit test was used to compare the residual and pure errors at the replicated design points. If the model fits the data well, lack of fit is not significant (as shown in Table III). Quadratic model was chosen as a highest-order significant polynomial having a non-significant lack of fit. The optimal conditions were predicted to obtain the highest reducing sugar yield and maximize enzymatic digestibility.

3.3 Optimization of the reducing sugars yield

The optimal values of each factor to optimize the process responses were based on Multi-Objective Numerical optimization [19]. The model equation for the response (Equ. 2), and the response surfaces (Fig. 2, A-C) were utilized in determining the optimum reducing sugars yield established by the pre-treatment conditions.

It was observed that only temperature as the main effect was significant. Temperature and time quadratic effects, all the factors interactions were also statistically significant on reducing sugars yield. In addition, the multiple correlation coefficients (R^2) of the regression equation obtained was about 92% for the reducing sugars yield, R^2 adjusted = 89%. These values mean the model for the reducing sugars response fitted well with the experimental data. The R^2-value implies that the sample variation of 92% is attributed to the factors, and also indicates that 8% of the total variation is not explained by the model. The ANOVA (Table III) for the regression model indicates that model is very significant as evident from the calculated *f*-value (30.980) and very low *P*- value ($p \leq 0.000$). Large *f*-value demonstrates that most of the variations in the response can be explained by the regression model equation.

The surface plots were used to determine the range of optimization. The response optimizer was maximized with lower value of 200 mg/g and upper value of 250 mg/g. The starting values of temperature, time, and H_2O_2 were kept at 70 ^oC, 5 h, and 1%(v/v) respectively.

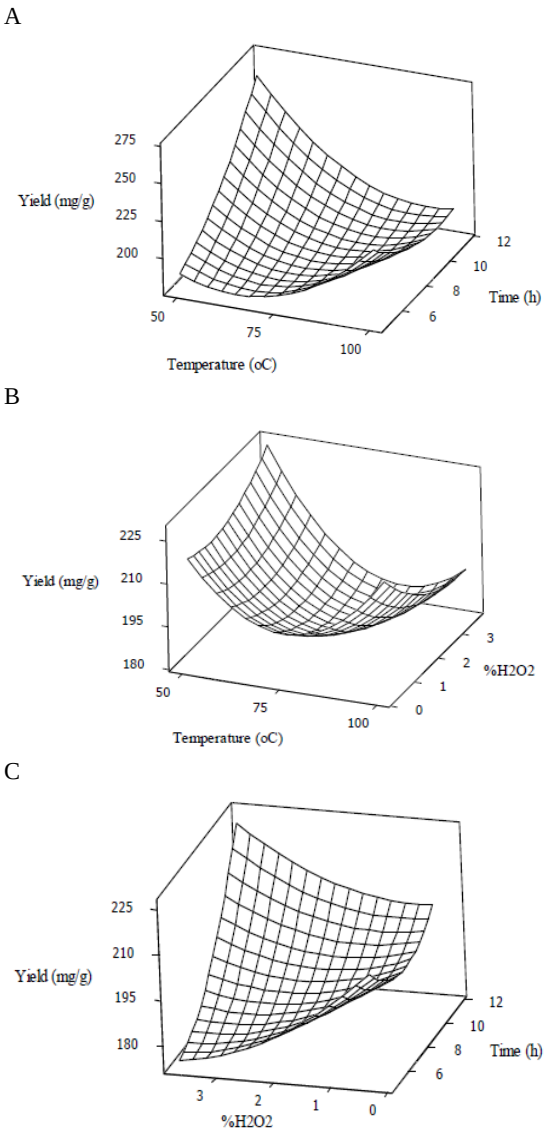


Figure 2: Surface plots of the responses for the reducing sugar yields (mg/g) of the enzymatic hydrolysis of pretreated rice husk versus (A) Temperature and Time; (B) Temperature and %(v/v) H_2O_2; (C) %(v/v) H_2O_2 and Time

The response value was 257.8 mg/g reducing sugar yield (desirability = 1.000) operating at the pretreatment conditions of 100 ^oC, 4.6 h, and 0.3%(v/v) H_2O_2. A validation of results from the model and regression equation was performed, which was evaluated to be 246.0 mg/g reducing sugar yield and compared with the predicted value. The predicted and validated responses

were found to be in close agreement, thus confirming the optimization process.

3.4 Variations of enzymatic digestibility at the optimized pretreatment conditions

At the optimized pretreament conditions, samples were subjected to varying amounts of substrate and enzyme loadings. Hydrolysis of untreated and washed biomass was also evaluated. The untreated solid material was used as the control for comparing the enzymatic digestibility of the treated rice husks. The 4-day reducing sugar yield (mg/g) of untreated and pretreated biomass were plotted against hydrolysis periods. The treated biomass were based on substrate loadings of 2%, 3%, 4%, and 5% (Fig. 3). Fig. 3 shows that it was necessary to treat the raw material before enzymatic saccharification. Pretreament causes a disruption in the lignocellulosic matrix thereby making the enzymes more accessible to substrates [20].

The sugar yields of the pretreated rice husks are significantly higher than the untreated. Results showed treated biomass maximum reducing sugars concentration of 271 mg/g for 96 h hydrolysis period (3% biomass loading) to untreated material of 64 mg/g reducing sugar yield at the same hydrolysis period. This was a 4-fold increase in reducing sugars produced from the treated to the untreated biomass. The enzyme loading study did not show any appreciable increase in reducing sugar yields (data not shown) from the value obtained at the optimized preatment conditions.

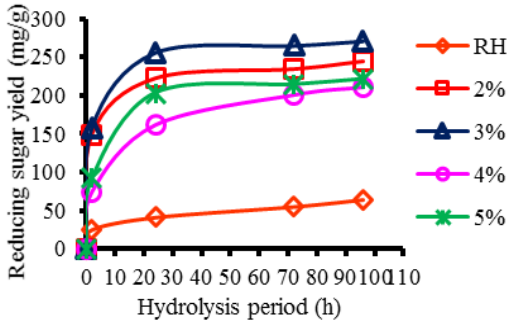


Figure 3: 4-d Effect of substrate loading on sugar yields. Pretreatment conditions: 100 ^{o}C, 4.6 h, and 0.3%H_2O_2. Enzyme hydrolysis conditions: 25 FPU enzyme loading per g dry biomass, 45 ^{o}C hydrolysis temperature, pH 4.8.
RH = Raw sample

3.5 Microscopy analysis of untreated, pretreated and enzyme digested biomass

3.5.1 Stereomicroscopy

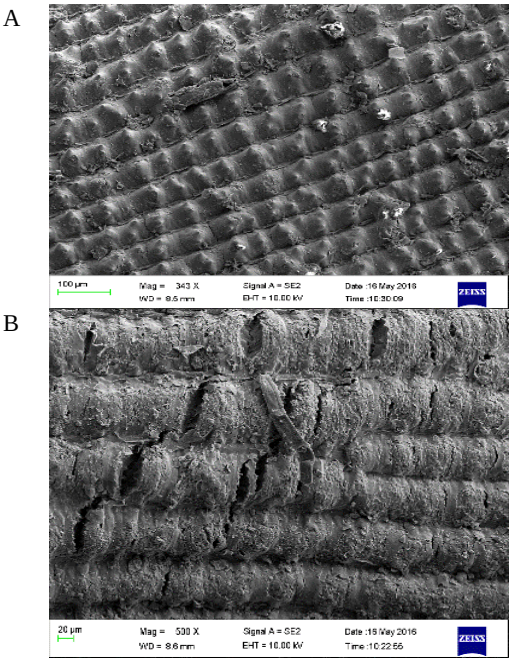
Stereoscope microscopy images and of the untreated, recovered solids at the optimized pretreatment conditions, and enzyme digested treated materials were analysed. Stereoscope micrographs of the untreated rice husks and residual solids following pretreatment and enzymatic digestibility are presented in Fig. 4. Stereoscope micrographs of biomass show the clumping of the particles caused by pretreatment and enzymatic digestibility. However, the pretreated samples appeared loosely packed than the enzyme treated samples. A trend of colour change from sandy brown (untreated) to yellow (pretreated) and then to clear yellow/light yellow (enzyme treated) was also observed.

Figure 4: Stereoscope micrographs taken at 1000 µm scale of the untreated (A), Pretreated (B), and enzymatic digested (C) rice husks

This shows the effect of hydrogen peroxide bleaching capacity coupled to the effect of NaOH on the biomass. The removal of chloropyll and plant cell cytoplasmic contents from the biomass materials was resposible for the colour changes [21]. The stereomicrography images also show a general trend of particle size reduction with increasing number of small and thin fibers for the pretreated and enzymatic hydrolyzed samples (Fig. 4).

3.5.2 Scanning electron microscopy (SEM)-Energy dispersive x-ray (EDX) diffraction

SEM images of the untreated, recovered solids at the optimized pretreatment conditions, and enzyme digested treated materials are shown in Fig. 5. Images show the well arranged structures in the raw sample (A) have been extremely made irregular by pretreatments and futher by the enzymatic hydrolysis. SEM displays cell wall distortion and the micro-fibres are pulled out from their coverings (A) and (B). The images further reveal significant surface distruptions especially in the enzyme digested pretreated samples.



C

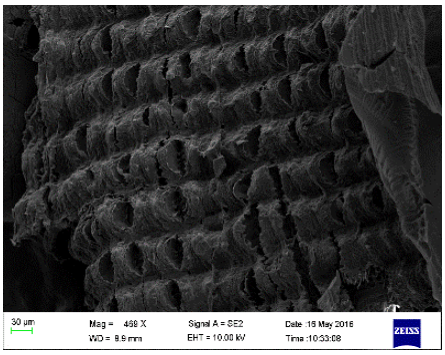


Figure 5: SEM micrographs showing changes to the inital raw rice husks (A), brought about by Pretreatment (B), and enzymatic hydrolysis (C)

EDX diffraction showed that carbon, oxygen and silicon are the main inorganic elements in all the samples (untreated, pretreated,and enzyme digested samples) (Table IV). Carbon and silicon compositions decreased from 40% and 18% respectively from raw to the enzyme digested sample. Oxygen composition increased to about 15% from the raw to the enzyme digested sample

Table IV: Elemental compositions of raw, pre-treated, and enzyme digested APO rice husks obtained by Energy dispersive x-ray (EDX) diffraction

Elements[c]	Raw	APO treated	Enzyme treated
C	31.82	29.34	18.78
	43.01	39.53	27.49
O	40.26	45.30	45.77
	40.83	45.82	50.31
Na	0.12	0.35	0.55
	0.08	0.25	0.35
Mg	0.08	0.63	0.90
	0.05	0.42	0.60
Si	27.45	23.22	22.66
	17.86	13.28	12.99
Al	0.26	0.63	0.10
	0.16	0.38	0.06

[c]First values of the elements represent the weight% while the second values represent the atomic%.

3.6 Spectroscopy analysis in the infrared region – FTIR

Information on the changes regarding chemical functionality can be observed fron the FTIR spectroscopy (Figure 6).

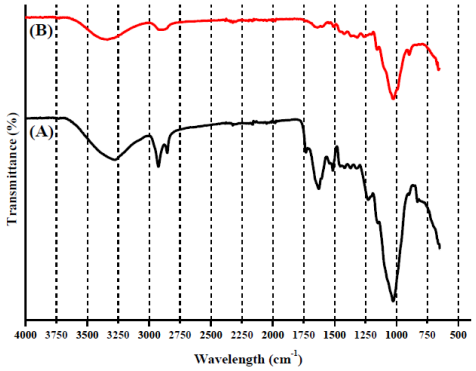


Figure 6: FTIR spectra of untreated (A), and pretreated enzyme digested (B) rice husks.

The FTIR spectroscopy reveals the structural analysis of the rice husks biomass before (Figure 6(A)) and after treatments (Figure 6(B)). The bands at 1595, 1510 and 1270 cm^{-1} are assigned to C=C, C–O stretching or bending vibrations of different groups present in lignin [22, 23]. The bands at 1460, 1425, 1335, 1220 and 1110 cm^{-1} are characteristic of C–H, C–O deformation, bending or stretching vibrations of many groups in lignin and carbohydrates [24, 25]. The bands at 1735, 1375, 1240, 1165, 1060 and 1030 cm^{-1} are assigned to C=O, C–H, C–O–C and C–O deformation or stretching vibrations of different groups in carbohydrates [22–24]. The degree of crystallinity in cellulose is related to TCI, and LOI measures the overall degree of order in the cellulose, while HBI is related to the crystal system and the degree of intermolecular regularity (crystallinity and the amount of bond waters) [15, 27–29]. Highest TCI and LOI values indicate highest degree of crystallinity and a more ordered cellulose structure. On the other hand, lowest TCI and LOI values indicate that the cellulose is composed of more amorphous structures [15].

The three ratios decreased from their initial values of the raw biomass to the digested samples, showing the effect of the cellulase enzymes on pretreated samples. From the calculated three ratios of the FTIR analysis, there were no significant differences betweeen the untreated and the enzyme digested samples (Table V). Many factors affect the enzyme digestibility of treated and untreated lignocellulosic biomass. The degradation of crystalline cellulose generally involves the action of both endo- and exo- acting cellulases [26]. Hydrolysis period as well as enzyme loadings are some of the factors that can affect the convertibility of treated lignocelluoses to bio-commodities as reducing sugars.

Table V: Infrared crystallinity ratio and hydrogen bond intensity of raw and enzyme treated biomass

	Infrared Crystallinity ratio		
Biomas	α_{1437} cm^{-1}/α_{899} cm^{-1} (LOI)	α_{1378} cm^{-1}/α_{2900} cm^{-1} (TCI)	α_{3400} cm^{-1}/α_{1320} cm^{-1} (HBI)
Raw	1.070	0.963	1.037
Enzyme digested	1.005	0.959	0.999

4 CONCLUSIONS

The effects of the major operational variables (temperature, time, and %H_2O_2) involved in the alkaline peroxide oxidation pretreatment process of rice husks, available to enzymatic hydrolysis, showed that appreciable amount of bio-commodities as reducing sugars can be produced. Established and validated optimum values were 100 ^oC, 4.6 h, and 0.3% (v/v) H_2O_2 to obtain maximum reducing sugar yield of 246 mg/g after hydrolysis period of 96 h, 45 ^oC hydrolysis temperature and 2% biomass loading. Increasing enzyme loading beyond 25 FPU/g biomass did not result in an increase in reducing sugar yield. An improvement in the reducing sugars production occurred by increasing the substrate loading from 2% to 3% during enzymatic digestion of the pretreated samples resulting in the production of 271 mg/g reducing sugar. The

spectroscopy analysis of the raw and treated samples in the infrared region showed very little deformation to the crystal system. Future works are to be directed in investigating various combinations of cellulose and hemi-cellulase enzymes that can improve the production of reducing sugars.

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7 LOGO SPACE