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Research Paper

Alkaline Pre-treatment and Enzymatic Saccharification of Rice husks for Bioethanol Production

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ABSTRACT
Aims: *This work was aimed at evaluating and optimizing the production of soluble sugars from bio-fermentation of rice husk using sodium hydroxide as a pre-treatment medium.*
Methodology: *Different samples of rice husk were pre-treated with 1%, 2%, 3% and 4% (w/v) sodium hydroxide respectively and allowed to de-lignify at various pre-treatment times of 2, 6, 8 and 24 hours before saccharification by cellulose at 50^{o}C and PH 4.8 for 48hours. Amount of reducing sugar produced was determined using spectrophotometric methods and recorded.*
Results: *High reducing sugar yields were recorded at 2%, 3% and 4%. NaOH were observed at 2% with a saccharification yield of 3.373 mg/ml, at 3% with a saccharification yield of 3.74 mg/ml and at 4% with a saccharification yield of 4.31mg/ml. The highest of reducing sugar yield was obtained at a NaOH concentration of 4.0(w/v) after a pre-treatment time of 24hours against other NaOH concentrations.*
Conclusion: *The result obtained suggests that the degree of saccharification is dependent on sodium hydroxide concentration used and time of pre-treatment. The reduction in reducing sugar yield at 1% NaOH may be due to NaOH solubilising lignin which may act as cellulose inhibitor during enzyme hydrolysis of the de-lignified rice husks.*
Key words: ***Rice husks, Lignocellulose, sodium hydroxide, saccharification, pre-treament, Fermentation.***

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I. INTRODUCTION

The increase in global population and industrialization and the continuous reliance on fossil fuel as a main source of energy, the increase in oil prices and dwindling global reserves has led to a search for alternative sources of energy [1,2].

Bio-ethanol can be produced from cellulosic biomass [3]. Bioethanol is a clean and renewable energy resource which does not contribute to global warming or environmental pollution. Product of industrial and agricultural wastes containing lignocellulosic proxide a promising source for renewable energy. They are the most abundant low-cost renewable resource worldwide [4]. The suitability of lignocellulosic substances for energy production is due do its abundance, accessibility, low cost of production and minimal impact on the environment [5]. Cellulose, Lignocellulose and lignin are the major constituents of lignocellulosic biomass. The major process of bioethanol production from lignocellulosic substrates involves various processing stages including pre-treatment (de-lignification) enzymatic saccharification and bio-fermentation of resulting sugars to produce bio-ethanol [6]. The structural complexity of lignin and hemi-cellulose play a significant role in affecting the alkaline hydrolysis of cellulose in fermentable sugars. Therefore an effective pre-treatment process is needed for the removal of lignin from biomass and the increasing the delignification and easy accessibility of cellulose to degradation by cellulose [7,8]. There are a number of pre-treatment methods including physical, biological and chemical [9, 10]. Among the chemical methods of pre-treatment, sodium hydroxide has been used and is found to be the most effective reagent for de-lignification of lignocelluloses in comparison to sulphuric acid or hydrogen peroxide [11].

When exposed to lignocelluloses content of rice husk, NaOh act by solubilising hemi-celluloses and lignin at neutral or weak acidic PH. Lignocellulosic biomass pre-treated with NaOH under de-lignification

resulting in improved digestibility [12]. Rice husk is a major by-product of agricultural processes. It is a cheap by-product of rice processing and is obtained during the milling process.

Approximately 700-800 million tonnes of rice husk is produced annually especially in Asia and Africa [13]. Here in Nigeria, most of the rice husk is burnt as waste while only a fraction (about 5%) is recycled for energy. [14,15]. This practice negatively impacts our environment. The huge availability and accessibility of rice husks provides promise for bio-ethanol production due to its carbohydrate content [16, 17].

To facilitate enzymatic hydrolysis of lignocelluloses biomass, the biomass undergoes physico-chemical pre-treatment which is essential for the removal of lignin and hemicelluloses, decreasing the crystaline structure of cellulose and increasing the porosity and hydrolisability of the biomass [18]. Various methods have been utilized in pre-treatment [9,12,18]. Pre-treatment of NaOH facilitates delignification due to an biomass with interaction between the aromatic ring of lignin with sodium hydroxide leading to increased digestibility [9].

In this research, the effect of varying concentration of sodium hydroxide and varying time on enzymatic hydrolysis of rice husk was invested and glucose formation was investigated and glucose formation was found to be pre treatment and enzyme-dependant.

II. MATERIALS AND METHODS

2.1 Materials

Cellulose enzyme from *Trichordema resei* ATCC26921 was bought from Zayo Sigma chemicals limited, Denmark. Sodium hydroxide, Dinitrosalicylic acide (DNS), Citrate monohydrate, D(+) glucose monohydrate crystalline phenol, Acetone, Tetracycline, cycloheximide, 70% ethanol, sodium metabisphosphate, sodium potassium tartrate, sodium citrate were purchased from City dealers and these reagents were of analytical grade.

2.2 Sample Collection

One kilogram (1kg) of rice husks was obtained from Miva rice mill in Makurdi, Benue State, Nigeria. The husks were transported to the biochemistry Laboratory, University of Agriculture, Makurdi. Nigeria for further analysis. The husks were sun-dried for 48hours, milled and stored in plastic bags at room temperature until used for pre-treatment.

2.3 Pre-treatment

Samples of dried, milled and sieved powdered rice husks were weighed into 20 different 250ml comical flasks stoppered with cotton wool. A set of four flasks were labelled 2 hours, 6hours, 8hours and 24hours respectively. 100ml of 1% NaOH was added to the four separate samples containing 10 g of rice husk each. The mixture was allowed to incubate for 2, 6, 8 and 24 hours respectively with continuous agitation for 20-30minutes. Samples were withdrawn at intervals of 2,6,8, 24hours and washed with distilled water, then filtered and the residue dried for 48hours. Dried samples were packaged in sealed plastic bag (LDPE) for further use.

2.4 Enymatic Saccharification

Enzymatic hydrolysis was carried out by the methods described by Fang et al, [19] using commercial cellulose enzyme purchased from Zayo Sigma [ZSA] chemical Ltd Denmark.

An amount, 4g of NaOH pre-treated rice husk was weighed into Sixteen 100ml conical flasks and another 4g untreated rice husk in another 100ml was set as control.

Also, 25ml 0.1M citrate buffer at pH 4.80 was measured and transferred into each flask to prevent microbial growth, 200µl of $10gml^{-1}$ tetracycline and $10gml^{-1}$ cyclohexamide in 70% ethanol was added to the samples. After this, 225ml of distilled water was also added and the system incubated at 100^0Cs for half an hour. The mixture was allowed to cool for 2hours before adding 1mg of the cellulose enzyme ATCC 26921 at a loading of 25FPu/g in order not to deactivate the enzyme. The enzymatic saccharification was carried out under mild conditions at PH 4.8 with addition of citrate buffer to maintain the pH and a temperature range of 40-50^0C which was kept constant in a photo-bioreator with enzyme loading of 1ml (25 FPU/g) for 48hours, then decanted and the supernatant centrifuged at 5000rpm for 30minutes. After which it was further subjected to spectrophotometric analysis.

2.5 Determination of reducing sugar concentration.

Reducing sugar content was estimated using 3,5-dinitrosalycilic acid (DNS) according to the method described by Miller[20].

Total reducing sugar concentration was determined spectrophotometrically using glucose as standard according to the method described by [21].

2.6 Determination of Reducing Sugar Yield

The reducing sugar yield was determined according to the method described by [22] using the equation shown below

Y = S x D x VW

Where

Y	=	Reducing Sugar yield from enzymatic hydrolysis (mg/g of dry biomass)
S	=	Sugar concentration in dilute sample (mg. Equivalent glucose/ml)

D = Dilution factor
V = Working Volume (ml)
W = Weight of dry biomass (g)

III. RESULTS

Table 1. Glucose concentration of rice husk hydrolysate at varying times and concentrations

Pre-treatment (Hour)	Dist. H_2O	Absorbance at varying % NaOH Concentrations					Reducing Sugar yield (mg/ml) at varying % NaOH concentrations				
		1%	2%	3%	4%	Contr	1%	2%	3%	4%	Control
2	0.15	0.087	0.486	0.822	1.050	ol0.0	0.45865	1.5775	2.98953	3.7143	0.3309
6	0.15	0.486	0.542	0.758	1.365	0.050	3.26124	2.0252	2.8435	4.8319	0.3309
8	0.15	0.880	0.986	1.070	1.037	0.050	3.1893	3.1554	3.8435	3.8636	0.3309
24	0.15	0.238	0.746	1.039	1.205	0.050	0.78378	2.7278	3.7368	4.3084	0.3309

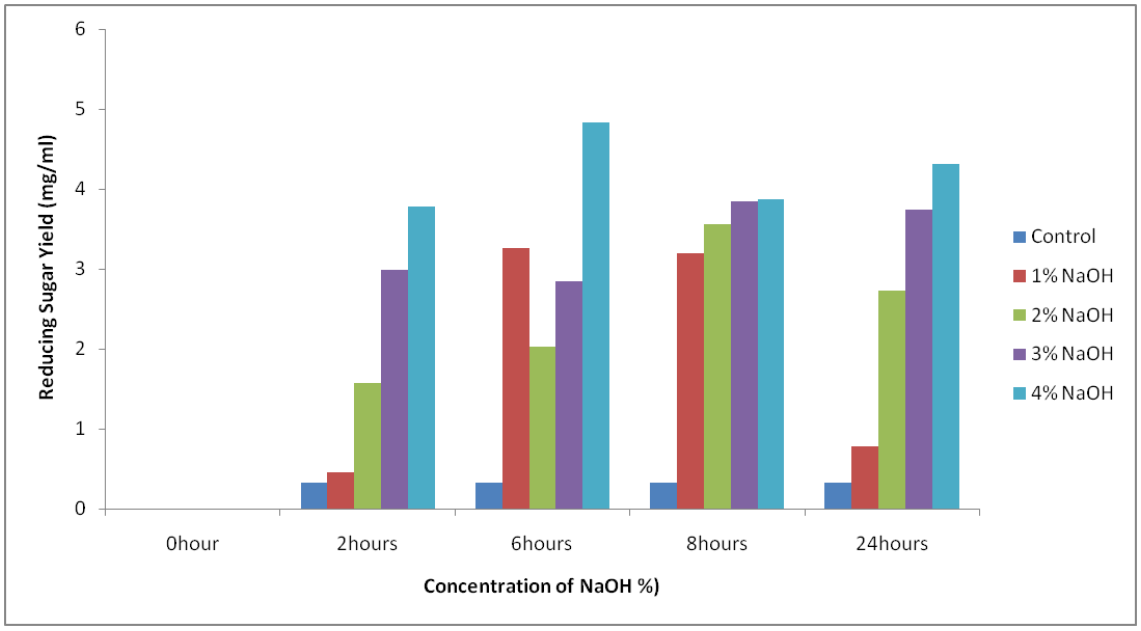

Figure 1 Bar chart of Reducing sugar yield (mg/ml) against the concentration of NaOH (%)

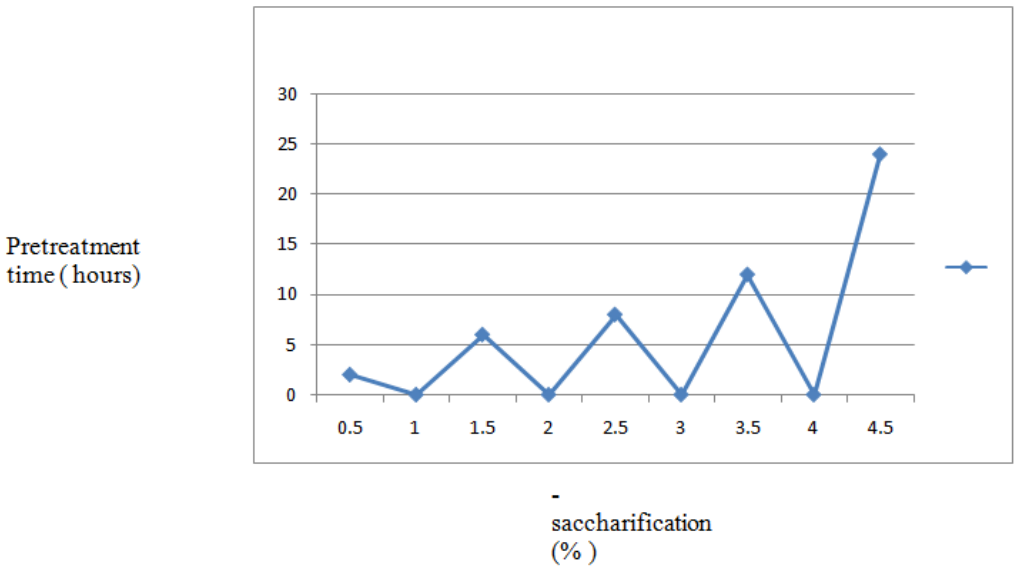

Fig 2:Plot of percentage saccharification against varying pretreatment time and concentration of NaOH solution.

IV. DISCUSSION

The effect of pre-treatment of rice husks using sodium hydroxide solution in percentage concentration on percentage reducing sugar (glucose) yield is shown in table 1figs. 1 and 2

After pre-treatment with varying concentrations of sodium hydroxide (1%, 2%, 3% and 4%) and enzymatic hydrolysis by cellulose enzyme, there was an increase in the amount of reducing sugars produced overall.

The amount of reducing sugars produced in the control (i.e. un-pre-treated sample) after the action of cellulose enzyme was constant with a value of 0.30309 mg/ml. After pre-treatment with NaOH for 2hours, the concentration increased to 0.45865, 1.577589, 2.989531 and 3.7743645 mg/ml at NaOH concentration of 1%, 2%, 3% and 4% (w/v) respectively.

Reducing sugar concentration was highest at 6hours of pre-treatment time for 2% and 4% (w/v) NaOH corresponding to 2.0252 and 4.8319 mg/ml respectively, after which there was a gentle drop in concentration of reducing sugars as can be seen in figure 1.

Higher concentration recorded agreed with the work of [9, 20] suggesting enhanced lignin solubilisation and decrease in cellulose crystalinity. Reducing sugar production was high when pre-treated with 4% NaOH concentration for 6hours which compared with other concentrations used. There was a recoded reduction in the yield of reducing sugars beyond 8 hrs of pre-treatment time. This may be due to the presence of solubilised lignin in the reaction mixture which may have affected the rate of enzyme activity [21].

The highest concentration of reduction sugar was recorded at 4% NaOH solution.Table 1 shows that glucose (simple sugar) yield in concentration is time and NaOH-concentration dependent. This present study suggests that Sodium hydroxide (NaOH) pretreatment of rice husks may help to increase the amount of reducing sugars after saccharification. This suggests that this work is in agreement with the work of Ikwebe which showed that alkaline (NaOH) pr-etreatment of biomass before enzymatic saccharification so as to have improved yield of reducing sugar.

V. CONCLUSION

The result of the research work shows that NaOH pre-treatment of rice cellulose may help to increase the amount of cellulose and resultant reducing sugars after enzymatic hydrolysis. Reducing sugar production was dependent upon the concentration of NaOH utilised for pre-treatment and the pre-treatment time. The highest value of sugar produced was 4.832 mg/ml at concentration time of 6 hours.

Conflict of interest

The authors of this work have no conflict of interest in so far this write- up is concerned.

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Authors contribution

The authors confirm contribution to the paper as follows: the corresponding author conceptualized and, designed and wrote the paper, author number 2 performed the analysis and contributed data or analysis tool.

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