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

^{1,2}*M.O. Nwankwo, ³J.J. Shirsha, ²D.C. Onah and ²D.Y. Lakabra

¹Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.

²Department of Biochemistry Joseph Sarwuan Tarka University Makurdi, Nigeria.

³Department of Nutritional Biochemistry, Benue State University, Makurdi, Nigeria.

*Correspondence E-mail: olykoh.17@gmail.com

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Abstract

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. 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 cellulase at 50 °C and pH 4.8 for 48 hours. The amount of reducing sugar produced was determined using spectrophotometric methods and was recorded. High reducing sugar yields 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 value of reducing sugar yield was obtained at a NaOH concentration of 4.0 (w/v) after a pre-treatment time of 24 hours against other NaOH concentrations. 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.

Keywords: Rice husks, sodium hydroxide, saccharification, pre-treatment, fermentation.

Introduction

The increase in global population, industrialization and the continuous reliance on fossil fuel as the main source of energy, the increase in oil prices and dwindling global reserves of oil has led to the search for alternative source(s) 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(s) of industrial and agricultural wastes containing lignocellulosic peroxide is 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 to 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 plays 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 the biomass and increasing the de-lignification and easy accessibility of cellulose to degradation by cellulase [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 results to 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 crystalline structure of cellulose and increasing the porosity and hydrolysis ability of the biomass [18]. Various methods have been utilized in pre-treatment [9,12,18]. Pre-treatment of NaOH facilitates delignification due to biomass interaction between the aromatic ring of lignin with sodium hydroxide leading to increased digestibility [9].

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

Materials and Methods

Materials

Cellulase from *Trichoderma reesei* ATCC26921 was bought from Zayo Sigma chemicals limited, Denmark. Sodium hydroxide, Dinitrosalicylic acid (DNS), Citrate monohydrate, D- glucose monohydrate crystalline phenol, Acetone, Tetracycline, cycloheximide, 98% ethanol, sodium meta-bisphosphate, sodium potassium tartrate, sodium citrate were purchased from Makurdi dealers and these reagents were of analytical grade.

Sample Collection

One kilogramme 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 48 hours, milled and stored in plastic bags at room temperature until they are used for pre-treatment.

Pre-treatment

Samples of dried, milled and sieved powdered rice husks were weighed into 20 different 250ml conical flasks stoppered with cotton wool. A set of four flasks were labelled 2, 6, 8 and 24 hours respectively. 100 ml 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 20-30 minutes. Samples were withdrawn at intervals of 2,6,8, 24 hours and washed with distilled water, then filtered and the

residue dried for 48 hours. Dried samples were packaged in sealed plastic bag (LDPE) for further use.

Enzymatic 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°Cs 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 ztemperature range of 40-50°C which was kept constant in a photo-bioreactor 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.

Determination of reducing sugar concentration.

The reducing sugar content was estimated using 3,5-dinitrosalicylic 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].

Determination of Reducing Sugar Yield

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

$$Y = S \times D \times VW \quad (1)$$

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)



Results and Discussion

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

Pre-treatment time (h)	Distilled water	Absorbance at varying % concs. of NaOH					Control	Reducing Sugar yield (mg/ml) at varying % NaOH concentrations					Control
		1%	2%	3%	4%			1%	2%	3%	4%		
2	0.15	0.087	0.486	0.822	1.050	0.050		0.4587	1.5775	2.9895	3.714	0.3309	
6	0.15	0.486	0.542	0.758	1.365	0.050		3.2612	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		0.7838	2.7278	3.7368	4.3084	0.3309	

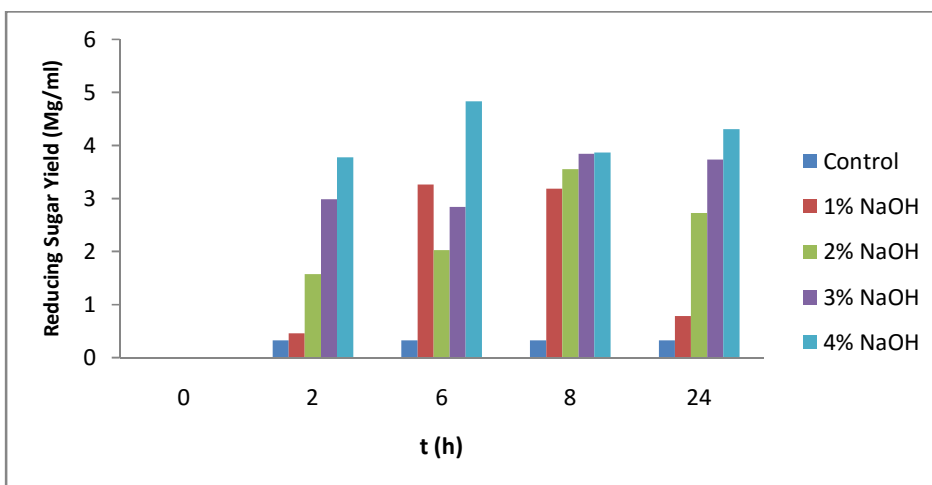


Figure 1 Reducing sugar yield (Mg/ml) against the concentration of NaOH (%)

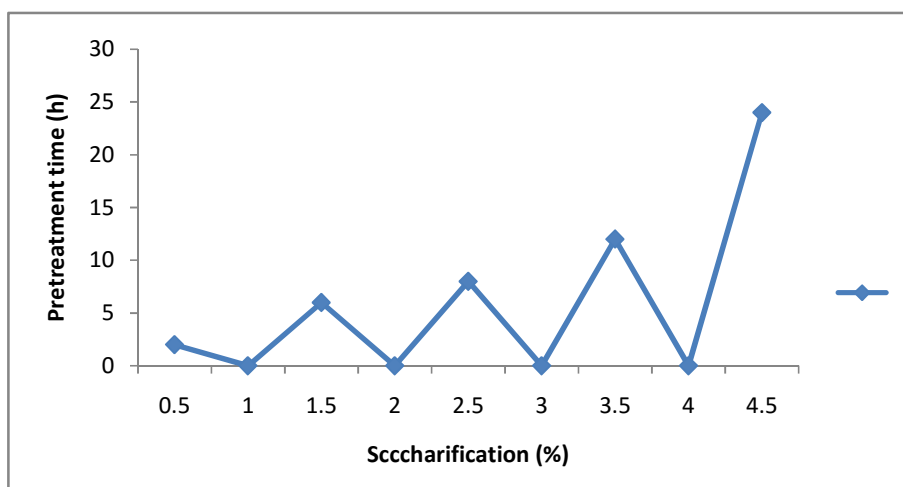


Fig 2: Plot of percentage saccharification against varying pretreatment Time and Concentration of NaOH solution.



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 1 figs. 1 and 2

After pre-treatment with varying concentrations of sodium hydroxide (1, 2, 3 and 4 %) and enzymatic hydrolysis by cellulase, 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 cellulase was constant with a value of 0.30309 Mg/ml. After pre-treatment with NaOH for 2 h, 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 6 hours 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.

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

Higher concentration recorded agreed with the work of [9, 20] suggesting enhanced lignin solubilisation and decrease in cellulose crystallinity. Reducing sugar production was high when pre-treated with 4 % NaOH concentration for 6 hours which compared with other concentrations used. There was a recorded reduction in the yield of reducing sugars beyond 8 hours 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 reducing 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) pre-treatment of biomass before enzymatic saccharification is to have improved yield of reducing sugar.

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 h.

Declaration of conflicting interests

The authors declared no potential conflicts of interest

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