***Short Research Article***

# Enzyme Hydrolysis of Hydrogen Peroxide Pretreated Lignocellulosic Content of Rice Husks Prior to Bioethanol Formation


## ABSTRACT

**Aims:** This work was aimed at evaluating the production of sugar from the bio-fermentation of rice husk using hydrogen peroxide as a pre-treatment medium.

**Methodology:** Different samples of rice husks were pretreated with 1%, 1.5%, 2%, and 2.5% (w/v) hydrogen peroxide respectively and allowed to delignify for 2 hours prior to saccharification by cellulose at $37^0$C and pH of 4.7. The experiment was repeated at varying time intervals spanning 2-24 hours. Amount of reducing sugars produced was determined spectrophotometrically and documented.

**Results:** High reducing sugar yields at concentrations of 1%, 1.5%, and 2%, $H_2O_2$ were observed as 0.81mg/ml with saccharification of 10.47%, 1.15mg/ml with saccharification of 14.87%, and 1.42mg/ml with saccharification of 18.36% respectively. Pretreatment with 2.0% $H_2O_2$ was found to achieve highest reducing sugars yield (1.42mg/ml with saccharification of 18.36%) after pretreatment time of 8 hours as against other concentrations.

**Conclusion:** The result suggests that degree of saccharification is dependent on concentration of hydrogen peroxide used and time of pre-treatment. The reduction in sugar yield at 2% $H_2O_2$ may be as a result of hydrogen peroxide solubilizing lignin which may act as an inhibitor during enzyme hydrolysis.



*Keywords: Agricultural wastes, Hydrogen Peroxide, Lignocelluloses, Pretreatment, Rice husks, Saccharification.*


## 1. INTRODUCTION

As a result of spike in world population and industrialization, continuous dependence on fossil fuels as a primary source of energy has led to an ever depleting natural resource and climate change. This continuous use of fossil fuels has led to environmental pollution and destruction of natural habitats [1]. In order to curb depletion of natural resources and continued pollution there has been an ever increasing demand for an alternative source of fuel [2]. Also, increasing cost of fuels for automobiles in recent years has led to a need to develop alternative sources of energy [3]. Ethanol can be produced from cellulosic biomass [4]. It is a clean and renewable energy resource and does not contribute to global warming.

Products of agricultural wastes containing lignocelluloses provide a promising source for renewable energy. Lignocellulosic is suitable for energy production due to its accessibility, low cost of production and small impact on the environment. Lignocellulose consists of lignin, hemicelluose and cellulose. The major process of bioethanol production from lignocellulosic biomass involves various processing stages which include; pre-treatment, enzymatic saccharification and subsequent fermentation of resulting sugars to produce ethanol [5]. The complexity in the structure of lignin and hemicelluloses play huge roles in affecting the hydrolysis of cellulose into fermentable sugars. An effective pre-treatment process is required for removal of lignin from biomass and increasing the accessibility of cellulose [6][7]. There are a number of pre-treatment methods including; physical, biological, and chemical [8][9]. Among the chemical method of pretreatment, hydrogen peroxide has been used and also sometimes in combination with alkali (NaOH) [10].

When exposed to biomass, $H_2O_2$ solubilize hemicellulose and lignin under alkaline or neutral conditions. Biomass pre-treated with $H_2O_2$ undergoes delignification leading to improved digestibility [11].

Rice husk is a major by-product of agricultural processes. It is an inexpensive byproduct of rice processing and is obtained during the processes of milling. Global rice production was estimated at 466 million tonnes in 2010 [12] with about 23% of this estimated to be made up of rice husks [13], majority of which is burnt or dumped as waste [14]. This practice negatively influences the environment. Rice husks provide a means for bio-ethanol production due to its carbohydrate content [15][16]. To facilitate enzyme hydrolysis of cellulose, biomass undergoes certain physical and physico-chemical pre-treatment. This pre-treatment is essential for the removal of lignin and hemicellulose, reduce cellulose crystalinity, increase porosity and enhance hydrolysability [17]. Previous works have shown that rice husks contain lignin, hemicellulose and cellulose [14]. This makes it a viable source for the production of bioethanol. Various methods have been utilized in pre-treatment [8][11][18]. Pretreatment of biomass with hydrogen peroxide facilitates delignification as a result of the interaction of the aromatic ring of lignin with hydrogen peroxide leading to increased digestibility [8]. In this work, the effect of different concentrations of hydrogen peroxide ($H_2O_2$) and varying time on enzyme hydrolysis was investigated and glucose formation was evaluated. To the best of our knowledge the effect of hydrogen peroxide pretreatment on the enzyme saccharification of rice husks have not been carried out in Nigeria.

## 2. MATERIAL AND METHODS

### 2.1 Materials

Cellulase extract from *Trichorderma reesei* was purchased from zayo Sigma (ZSA) Chemical Ltd Denmark. Dinitrosalicylic acid reagent (DNS), citrate monohydrate, D (+) glucose monohydrate, hydrogen peroxide was purchased from dealers and was of analytical grade.

### 2.2 Sample Collection

Five hundred (500g) grams of rice husks was collected from Milva rice mill in Makurdi, Benue State. The rice husks were taken to the Biochemistry lab, University of Agriculture, Makurdi, Nigeria for further analysis. The husks were shade dried at room temperature, crushed into powder and stored in plastic bags until use for pre-treatment.

### 2.3 Pre-treatment

Samples of powdered rice husks were weighed into 20 different 250ml conical flasks. To a set of four flasks labelled 2hr, 6hr, 8hr and 24hr, 100ml of 1% (w/v) hydrogen peroxide dissolved in distilled water was added respectively, to represent group 1. The solid to liquid ratio was 1:10. The same procedure was repeated at 1.5%, 2.0%, and 2.5% Hydrogen peroxide representing groups 2, 3, and 4 respectively. Samples of powdered rice were also dissolved in 100ml of distilled water to represent control group. The resulting samples were stored for incubation for the duration of time as labelled for each tube. The pretreatment was carried out at a temperature of $35^0$C.

### 2.4 Enzyme Hydrolysis

Hydrolysis was carried out by the method described by Fang *et al.,* [19], using commercial cellulase enzyme purchased from Zayo Sigma [ZSA] Chemical Ltd Denmark.

Briefly, pretreated samples were weighed in separate 30ml test tubes and labelled accordingly, followed by addition of 15ml citrate buffer (pH 4.5) and 200µl of tetracycline and griseofulvine (prepared by dissolving 500mg of each in a combined volume of 100ml of 70% ethanol). Sterile distilled water was added to make up to the mark, and 0.5m of cullulase enzyme was added and stirred at intervals of 48hrs at $37^0$C and pH of 4.8 After filtration and centrifugation, hydrolysates were collected in separate sample tubes labelled according to the content of the pre-treated samples.

### 2.5 Determination of Reducing Sugar Concentration

Reducing sugar content was estimated according to the method of Miller [20], as described by Anamaria [21]. The percentage saccharification was calculated using the equation of Mendels and Sternberg [22] as shown by Alrumman [23]

$$\%saccharification = \frac{Reducing\ sugars\left(\frac{mg}{ml}\right)\times 0.9\times 100\%}{initial\ substrate\ concentration\ \left(\frac{mg}{ml}\right)}$$

## 3. RESULTS AND DISCUSSION

The effect of pre-treatment of rice husks using hydrogen peroxide dissolved in water (w/v) on glucose concentration and percent (%) saccharification is shown below in Table 1; Fig 1 and 2. After pre-treatment with varying concentrations of hydrogen peroxide (1%, 1.5%, 2% and 2.5%) and hydrolysis by cellulase enzyme, there was an increase in the amount of reducing sugars produced. The amount of reducing sugars produced in the un-pretreated sample after the action of cellulase enzyme was fairly constant, with a value of 0.16mg/ml. After pre-treatment with hydrogen peroxide for two hours, the concentrations were increased to 0.35, 0.77, 1.03 and 0.25mg/ml (Fig 1) with saccharification of 4.5, 9.96, 13.32, and 3.23% (Fig 2) at hydrogen peroxide concentrations of 1%, 1.5%, 2% and 2.5% (w/v) respectively. Reducing sugar concentration was highest at 8 hours of pre-treatment time for 1%, 1.5% and 2% (w/v) $H_2O_2$ corresponding to 0.81, 1.15, 1.42mg/ml with saccharification of 10.47, 14.87 and 18.36% respectively after which, there was a drop in concentration of reducing sugars as can be seen in Fig 1. Highest concentration recorded at 2.5% $H_2O_2$ was observed at the 24 hour mark corresponding to 0.57mg/ml and a saccharification of 7.37% (Fig 2). It was observed that percent saccharification increased with increase in concentration of hydrogen peroxide which agrees with the works of Diaz *et al.,* [10].

The findings in this work demonstrate that pre-treatment with hydrogen peroxide induced production of cellulose which was subsequently hydrolysed into reducing sugars. Concentration of reducing sugars continues to increase with increasing concentration of hydrogen peroxide as peroxide was increased from 1% to 2%. This is in agreement with the works [10] and [24], suggesting enhanced lignin solubilisation and decrease in cellulose crystalinity. Reducing sugars produced were high when pre-treated with 2% $H_2O_2$ for 8 hours when compared with other concentrations used. Concentration of reducing sugars decreased when measured after 24 hours of pretreatment. The reduction in yield over time and at 2.5% $H_2O_2$ may be as a result of production of soluble lignin compounds [11] which may interfere with generation of fermentable sugars.
This work suggests that hydrogen peroxide may serve as an option for pre-treatment of rice husks for the production of fermentable sugars as has been suggested by earlier findings of Shen *et al.,* which stated that pretreatment of biomass with $H_2O_2$ before enzymatic hydrolysis was effective in improving cellulose recovery, removal of lignin and improving reducing sugar yield [24].

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

| Pre-treatment(hrs) | Reducing sugars | | | | |
|---|---|---|---|---|---|
| | Concentration of $H_2O_2$ | | | | |
| | Dist $H_20$ | 1.0 (W/V) | 1.5 (W/V) | 2.0 (W/V) | 2.5 (W/V) |
| | Mg/ml | Mg/ml | Mg/ml | Mg/ml | Mg/ml |
| 2 | 0.16 | 0.35 | 0.77 | 1.03 | 0.25 |
| 6 | 0.16 | 0.46 | 0.79 | 1.04 | 0.46 |
| 8 | 0.16 | 0.81 | 1.15 | 1.42 | 0.46 |
| 24 | 0.16 | 0.37 | 0.38 | 0.75 | 0.57 |

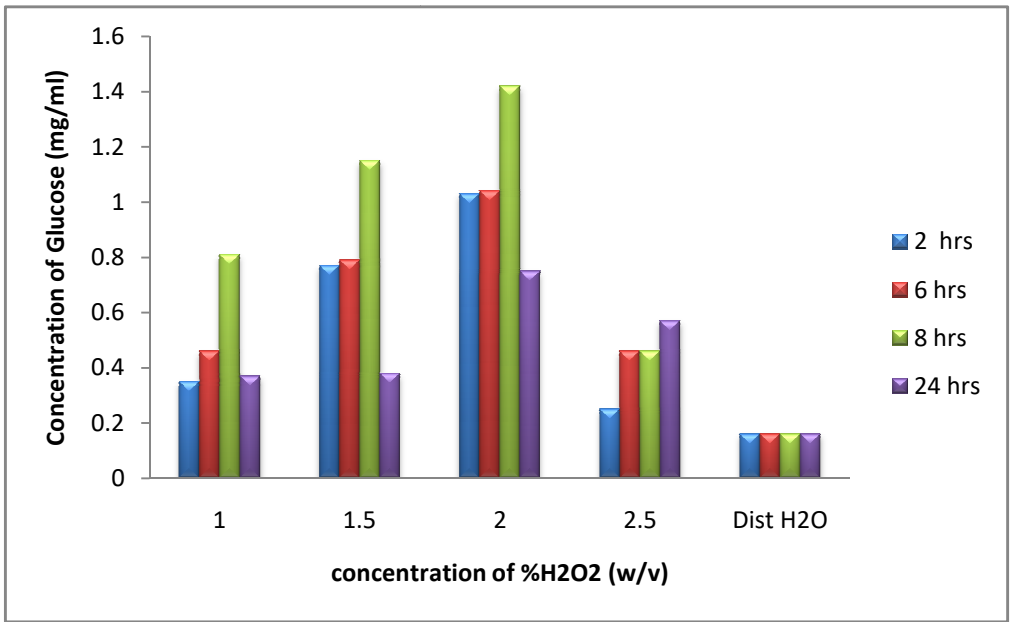


**Fig 1. Bar chat of concentration of glucose against different concentrations of hydrogen peroxide and varying pre-treatment time.**

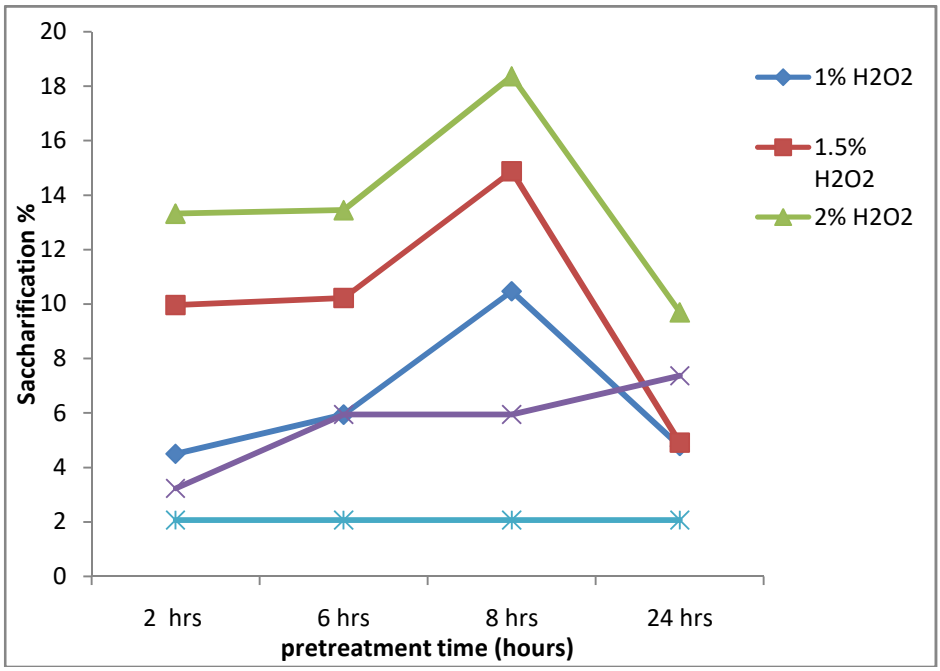


**Fig. 2 Plot of percent saccharifiction against varying pretreatment time and different hydrogen peroxide concentration**

## 4. CONCLUSION

The results of this investigation shows that $H_2O_2$ pretreatment of rice husks may help to increase the amount of cellulose and resultant reducing sugars after enzymatic hydrolysis. Sugar production was dependent of concentration of hydrogen peroxide and time of pretreatment. The highest value for sugars produced was 1.42mg/ml with saccharification of 18.36% at pretreatment time of 8 hours and 2% $H_2O_2$ concentration. Amount of sugar produced was lower after pretreatment conditions of 2.5% $H_2O_2$ when compared with other concentrations. Further experiments needs to be carried out to investigate the effect of temperature on yield.

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