

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°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%, H₂O₂ 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. Pretreatmet with 2.5% H₂O₂ achieved highest reducing sugars yield (0.57mg/ml with saccharification of 7.37%) with pretreatment time of 24 hours as against other concentrations with optimal yields at the 8 hour mark.

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% H₂O₂ may be as a result of hydrogen peroxide solubilising 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 cellulosaiac 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 consist of lignin, hemicelluose and cellulose. The major process of bioethanol production from lignocellulosic biomas 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 solubilise 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 hemicelluloses, reduce cellulose crystallinity, increase porosity and enhance hydrolysability [17]. 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.

2. MATERIAL AND METHODS

2.1 Materials

Cellulase extract from *Trichoderma 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 Markurdi, 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 the 2 grams 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. To another set of four flasks labelled as described above, 1.5% Hydrogen peroxide was added to represent group 2. The same procedure was repeated for 2% and 2.5% hydrogen peroxide respectively. The resulting samples were stored for incubation for the duration of time as labelled for each tube.

2.4 Enzyme Hydrolysis

Hydrolysis was carried out by the method described by Fang *et al.*, [18], 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 200 μ l 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 0.5ml of cellulase enzyme was added and stirred at intervals of 48hrs at 37°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 [19], as described by Anamaria [20].

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) H₂O₂ respectively. Reducing sugar concentration was highest at 8 hours of pre-treatment time for 1%, 1.5% and 2% (w/v) H₂O₂ 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% H₂O₂ was observed at the 24 hour mark corresponding to 0.57mg/ml and a saccharification of 7.37% (Fig 2). It was observed that % saccharification increased with increase in concentration of hydrogen peroxide which agrees with the works of Diaz *et al.*, [10].

Previous works have shown that rice husks contain lignin, hemicelluloses and cellulose [14]. This makes it a viable source for the production of bioethanol. For cellulose present in rice husks to be utilized for bioethanol production, the rice husks must first be processed. One such process is the pre-treatment of biomass to facilitate the removal of lignin and to disrupt the crystalline structure of cellulose to enable adequate hydrolysis by cellulase enzyme. Various methods have been utilized in pre-treatment [8][11][21].

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 [22], suggesting enhanced lignin solubilisation and decrease in cellulose crystallinity. Reducing sugars produced were high when pre-treated with 2% H₂O₂ for 8 hours when compared with other concentrations used. Concentration of reducing sugars decreased when measured after 24 hours. The reduction in yield over time and at 2.5% H₂O₂ 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 H₂O₂ before enzymatic hydrolysis was effective in improving cellulose recovery, removal of lignin and improving reducing sugar yield [22]. Other investigators who also suggest the positive effect of hydrogen peroxide on cellulose recovery include [23][10].

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

Pre-treatment(hrs)	Reducing sugars				
	Concentration of H ₂ O ₂				
	Dist H ₂ O	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

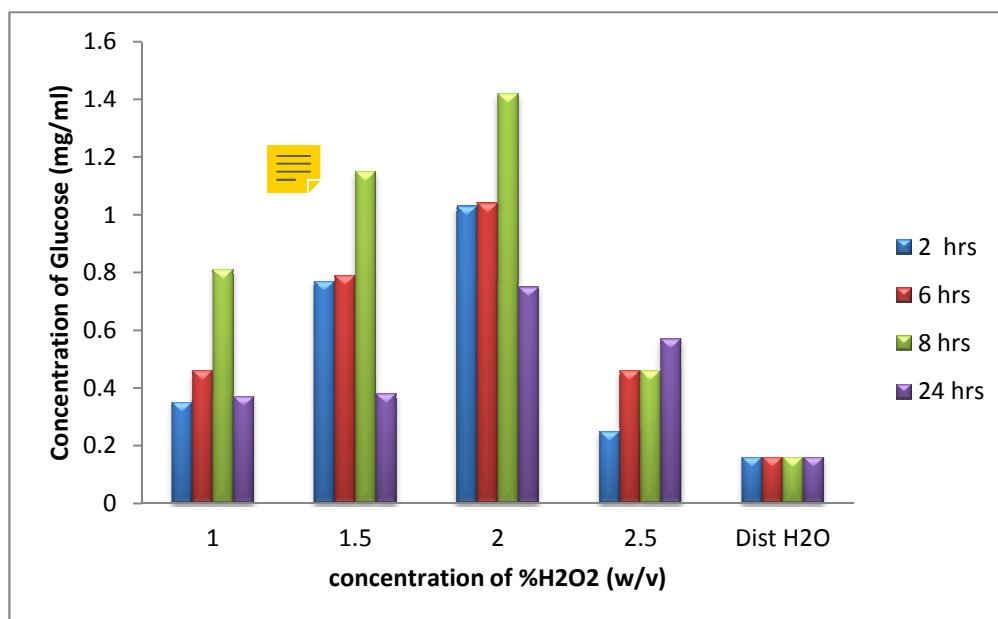


Fig 1. Bar chat of concentration of glucose against concentration of hydrogen peroxide with pre-treatment time.

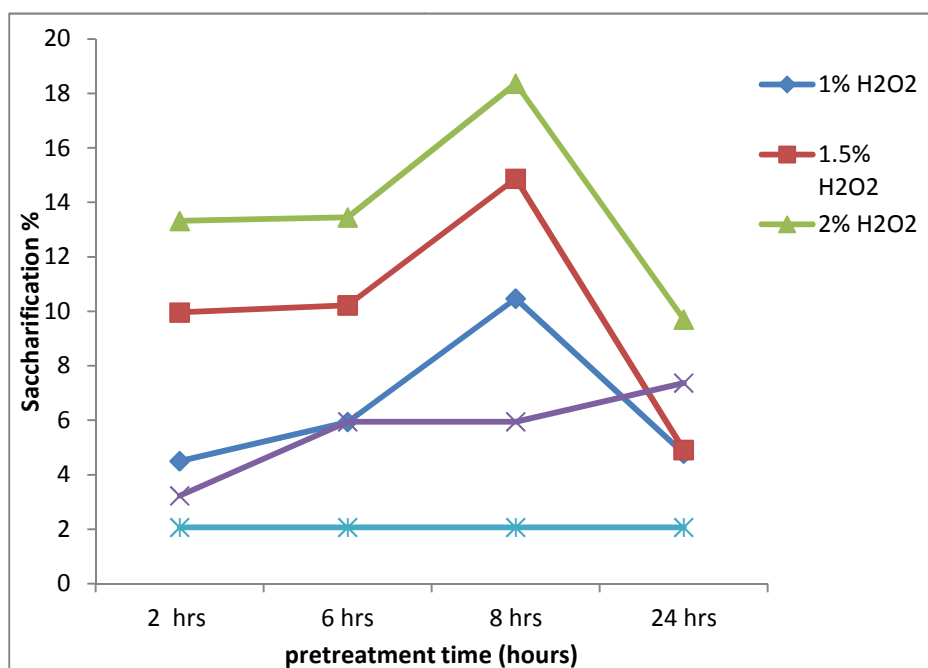


Fig. 2 Plot of % saccharification against pretreatment time

4. CONCLUSION

The results of this investigation shows that H₂O₂ 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% H₂O₂ concentration. Amount of sugar produced was lower after pretreatment conditions of 2.5% H₂O₂ when compared with other concentrations. Further experiments needs to be carried out to investigate the effect of temperature on yield.

REFERENCES

1. Hirsch, S.; and Rittner, F. Geothermal energy development- Possible market facilitation roles of UNEP and GEF. In: *Geothermal energy resource for developing countries* 2002. Eds: Chandrashekharan, D., Bundschuh, J. Swets and Zeitlinger B.V. Liesse, The Netherlands 3: pp. 91-102.
2. Louime, C.; and Uckelmann, H. Cellulosic ethanol: Securing the planet future energy needs, *Int. J. Mol. Sci.* 2008, 9, pp. 838-841,
3. Moiser, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y.Y.; and Holtzapple, M. Features of Promising Technologies for Pretreatment of Lignocellulosic Biomass. *Bioresour. Technol.* 2005, 96, pp. 673-686,
4. Masud, H.Sk.; Anantharaman, N.; and Manas, D. Bioethanol Fermentation from Untreated and Pretreated Lignocellulosic Wheat Straw Using Fungi *Fusarium oxysporum*. *Indian J.Chem. Technol.* 2012, 19, pp. 63-70,
5. Gómez, S.M.R.; Andrade, R.R.; Santander, C.G.; Costa, A.C.; Maciel, F.R. Pretreatment of sugar cane bagasse with phosphoric and sulfuric diluted acid for fermentable sugars production by enzymatic hydrolysis, *Chem. Eng. Trans.* 2010, 20, pp. 321-326.
6. Sun, Y.; and Cheng, J. Hydrolysis of lignocellulosic materials for ethanol production: a review, *Bioresour. Technol.*, 2002, vol.83, no. 1, pp. 1–11.
7. Persson, T.; Ren, J.L.; Joelsson, E.; and Jönsson, A.S. Fractionation of Wheat and Barley Straw to Access High-Molecular-Mass Hemicelluloses Prior to Ethanol Production, *Bioresour. Technol* 2009, vol. 100, no. 17, pp. 3906–3913.
8. Bensah, E.C.; Mensah, M. (2013). "Chemical pretreatment methods for the production of cellulosic ethanol: Technologies and Innovations", *Int. J. Chem. Eng.* 2013, doi: 10.1155/2013/719607
9. Yang, C.Y.; Sheih, C.; Fang, T.J. Fermentation of rice hull by *Aspergillus japonicus* under ultrasonic pretreatment, *Ultrason. Sonochem.* 2012, 9, pp. 687-691.
10. Diaz, A.; Toullec, J.L.; Blandino, A.; Ory, I.D.; Caro, I. pretreatment of rice hulls with alkaline peroxide to enhance enzyme hydrolysis for ethanol production. *Chem. Eng. Trans.* 2013, 32, pp. 949-954.
11. Harmsen, P.; Huijgen, W.; Bermudez, L.; Bakker, R. Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. Tech. Rep. 1184, Biosynergy,Wageningen UR Food & Biobased Research, 2010.
12. Food and Agricultural Organization. "Global cereal supply and demand brief. Crop prospects & food situation" (no. 4, pp. 5, (Dec, 2010)). <http://www.fao.org/giews/>
13. Chandrasekhar, S.; Satyanarayana, K.G.; Pramada, P.N.; Raghavan, P.; Gupta, T.N. Processing, properties and applications of reactive silica from rice husk – An overview. *J. Mater. Sci.* 2003. 38, pp. 3159–3168.
14. Ajay, K.; Kalyani, M.; Devendra, K.; Om P, P. Properties and industrial applications of rice husk: A review. *Int. J. Emer. Technol. Adv. Eng.* 2012. vol 2 no.10, pp. 86-91.
15. Martin, C.; Lopez, Y.; Plasencia, Y.; Hernandez, E. Characterisation of agricultural and agro-industrial residues as raw materials for ethanol production. *Chem. Biochem. Eng. Q.* 2006, vol. 20 no. 4, pp. 443-446.
16. Martin, C.; Alriksson, B.; Nilverbrant, N.O.; Jönsson, L.J. Dilute sulphuric acid pretreatment of agricultural and agro-industrial residues for ethanol production. *Appl. Biochem. Biotechnol.* 2007, 136/140, pp. 339-352.

17. Tarhezadeh, M.J.; Karimi, K. Enzyme-based hydrolysis process for ethanol from lignocelulosic materials: A Review. *BioRes.* 2007b, vol. 2 no. 4, pp. 707-738.
18. Fang, H.; Zhao, C.; Song, X. Optimization of enzymatic hydrolysis of steam exploded corn stover by two approaches: responsesurface methodologyor using cellulase from mixed cultures of *Trichoderma reesei* RUT-C30 and *Aspergillus niger* NL02. *Bioresour. Technol.* 2009, vol. 101, pp. 4111-4119.
19. Milller, G.L. Use of Dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 1959; vol. 31, no. 3 pp.426-428.
20. Anamaria, N.; Viorica, P.; Manuela, M.M.; Cosmin, A.; Beatrice, V.; Ostafe, V. Adapting the reducing sugars method with dinitrosalicylic acid to microtiter platesand microwave heating. *J. Braz. Chem. Soc.* 2012, vol. 23, no. 12, pp. 2176-2182.
21. Binod, P.; Sindhu, R.; Singhanian, R.R.; Vikram, S.; Devi, L.; Nagalakhmi, S.; Kurien, N.; Sukumaran, R.K.; Pandey, A. Bioethanol production from rice straw: an overview. *Bioresour. Technol.* 2010. Vol. 101, pp. 4767-4774.
22. Shen, G.; Tao, H.; Zhao, M.; Yang, B.; Wen, D.; Yuan, Q.; Rao, G. Effect of hydrogen peroxide pretreatment on enzymatic hydrolysis of cellulose. *J. food process.* 2009. Vol. 34, no. 3, pp. 905-921.
23. Kim, S.B.; Um, B.H.; Park, S.C. Effect of pretreatment reagent and hydrogen peroxide on enzymatic hydrolysis of oak in percolation process. *Appl. Biochem. Biotechn.* 2001, 91-93:pp. 81-94.