

Original Research Article

ENZYMATIC CONVERSION OF WASTE (RICE HUSK) INTO FERMENTABLE SUGAR THROUGH DILUTED ACID PRETREATMENT

Abstract

The enzymatic production of fermentable sugar from rice husk was examined using diluted sulfuric acid as a pretreatment medium. In this study, 10g of rice husk were separately pretreated with 0.5M, 1.0M and 1.5M sulfuric acid at different time interval of 2-24hrs prior to saccharification by cellulase enzyme at 37°C and pH 4.5. Then the quantity of monosaccharide produced was determined spectrophotometrically. After two hours of pretreatment, the 1.5M H₂SO₄ pretreated samples yielded 88.05 mg/g (39.11%) of glucose, 1.0M H₂SO₄ pretreated samples yield 68.4mg/g (30.38%), and 0.5M H₂SO₄ pretreated samples yield 68.7mg/g (30.51%) respectively, when compared with the un-pretreated sample (20.4mg/g). However, after two hours, the production of glucose significantly reduces with increasing time, except with 1.0 and 0.5M H₂SO₄ pretreated sample which significantly increases at 4 hours (82.5 and 76.5mg/g respectively). The result suggests that sulfuric acid enhances the release of fermentable sugar, and that the yield is dependent on the concentration of acid and the time of treatment. The reduction in glucose yield over time as shown in the results could be due to the ability of the acid to liberate sugar which may be washed off during the washing process to remove the acid prior to the hydrolysis by enzyme.

Key words: lignocellulose, pretreatment, saccharification, rice husk, enzyme

1. Introduction

Fermentable sugars are important prerequisite for ethanol production, and they are insufficient for production of the required amount of ethanol, in spite of the abundance of raw materials [1, 2].

There has been an un-ending debate over bioethanol produced from food crops and future food security [3]. Although at the moment the bioethanol produced by a nation is dependent on the prevalent feedstock (for example, sugarcane for Brazil, corn for USA and cassava for Nigeria), it is increasingly understood that 1st-generation bioethanol produced primarily from food crops is limited in its ability to achieve targets for oil-product substitution, climate change mitigation and economic growth [4, 5]. The sustainable production of these fuels is still currently under review, as is the possibility of creating undue competition for land and water used for food and fiber production [6]. A possible exception that appears to meet many of the acceptable criteria is ethanol produced from sugar cane and cassava [4]. These concerns have accelerated interest in developing bioethanol produced from non-food biomass [7, 8, 9].

Research efforts have been focused on designing economically viable processes capable of sustainably producing high amounts of fuel bioethanol. The cost effectiveness of bioethanol production through hydrolysis of starchy substrates by using enzymatic/microbial processes has been proved commercially viable [10]. The main sources of sugar required to produce ethanol come from fuel or energy crops, which include maize, cassava and cassava products, wheat crops, waste straw, guinea corn husk, rice husk, millet husk sawdust and sorghum plant. However, rice husk ash is a great environment threat causing damage to land and surrounding area where it is dumped [11, 12].

Rice husk (RH) is one of the most widely available agricultural wastes in many rice producing countries around the world. It contributes to about 20% of the weight of rice [13]. Globally, approximately 600 million tons of rice paddy is produced each year. On average 20% of the rice paddy is husk, giving an annual total production of 120 million tones [7]. In majority of rice

producing countries much of the husk produced from processing of rice is either burnt or dumped as waste [14], hence the need for a clean energy technology throughout the world to meet energy demand due to decline in fossil fuel [15, 16].

Rice husk removal during rice refining, creates disposal problem due to less commercial interest. Also, handling and transportation of RH is problematic due to its low density. RHA is a great environmental threat causing damage to land and surrounding area where it is dumped. Therefore, commercial use of rice husk and its ash is the alternative solution to disposal problem. Rice husk contains 75-90 % organic matter such as cellulose, lignin etc. and rest mineral components such as silica, alkalis and trace elements [14].

Pretreatment is a crucial process step for the biochemical conversion of lignocellulosic biomass into fermentable sugar [17, 18]. It is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars [19, 20]. Possible goals include the removal of lignin and disruption of the crystalline structure of cellulose [8, 19, 21]. Though many work has been ongoing in this area [22, 23], to the best of our knowledge none has utilized room temperature treatment of lignocellulose.

2. Materials and Methods

2.1. Materials/Chemicals: Cellulase extracted from *Trichoderma reesei*, was purchased from Zayo Sigma [ZSA] Chemical Ltd Denmark. Dinitrosalicylic acid reagent (DNS), citrate monohydrate, D(+) glucose monohydrate, sodium hydroxide, Sulfuric acid were purchased from the dealers and were of analytical grades.

2.2. Samples Collection: Four hundred gram (400g) of rice husks were collected from Milva rice mill dump site in Makurdi, Benue State. They were aseptically collected in polythene bag and taken to the Biochemistry laboratory, University of Agriculture, Makurdi, Nigeria for further analysis.

The husk was shade dried at room temperature, and milled into powdered form. The powder obtained was subjected to pretreatment options.

2.3. Pre-treatment Protocol: Ten grams of the powdered sample was weighed into 20 different 250ml conical flasks. To a set of five flasks labeled 2hr, 4hr, 6hr, 8hr and 24hr, 90mL of 0.5 M sulfuric acid was added respectively, to represent group 1. To another set of five flasks labeled 2hr, 4hr, 6hr, 8hr and 24hrs, 90mL of 1.0 M sulfuric acid was added to represent group 2. The third set of five flasks labeled 2hr, 4hr, 6hr, 8hr and 24hrs, 90mL of 1.5 M sulfuric acid was added to represent group 3. For same incubation time, a set of control was set up using distilled water.

Each group mixture was allowed to stand. After two hour the flasks labeled 2hr were decanted and the pretreated samples washed several times with distilled water to remove the remaining acid solution, and subjected to air-dry in a sterile petri dish. The same method was applied to the 4hr, 6hr, 8hr, and 24hr labeled preparations. The resulting dried pretreated samples were stored and labeled accordingly [24].

2.4. Enzyme Hydrolysis: Enzymatic hydrolysis was carried out by method described by [25], using commercial cellulase enzyme, purchased from Zayo Sigma [ZSA] Chemical Ltd Denmark.

Two grams of each pretreated sample was weighed inside separate 30mL test tubes and labeled accordingly, followed by addition of 15 mL citrate buffer (pH 4.5) and 200 μ L of tetracycline and griseofulvine (prepared by dissolving 500 mg of each in a combine volume of 100 mL of 70% ethanol). Sterile distilled water was added to make up to the mark, and 0.5 mL of cellulase enzyme was added and stirred at intervals for 48hrs at 37⁰C and pH 4.8.

After filtration and centrifugation, the hydrolysate was collected in separate sample tubes labeled according to the condition of pre-treatment.

2.5. Determination of Reducing Sugar Concentration: The reducing sugar content in the hydrolysates was estimated according to the protocol of [26], as modified by [27].

3. Result and Discussion

The plot of concentration of glucose against time of pretreatment is presented in Figure 1 and 2. In this study, sulfuric acids were used to pretreat the polysaccharide content of rice husk, followed by saccharification of the polysaccharide by cellulose. From the results, the total soluble sugar concentrations increased to 88.05, 68.4, and 68.7mg/g upon pretreatment with 1.5, 1.0 and 0.5M H₂SO₄ respectively, when compared with the un-pretreated sample (20.4 mg/g). However, after two hours, the production of glucose significantly reduces with increasing time, except with 1.0 and 0.5M H₂SO₄ pretreated sample which significantly increases at 4 hours (82.5 and 76.5mg/g respectively).

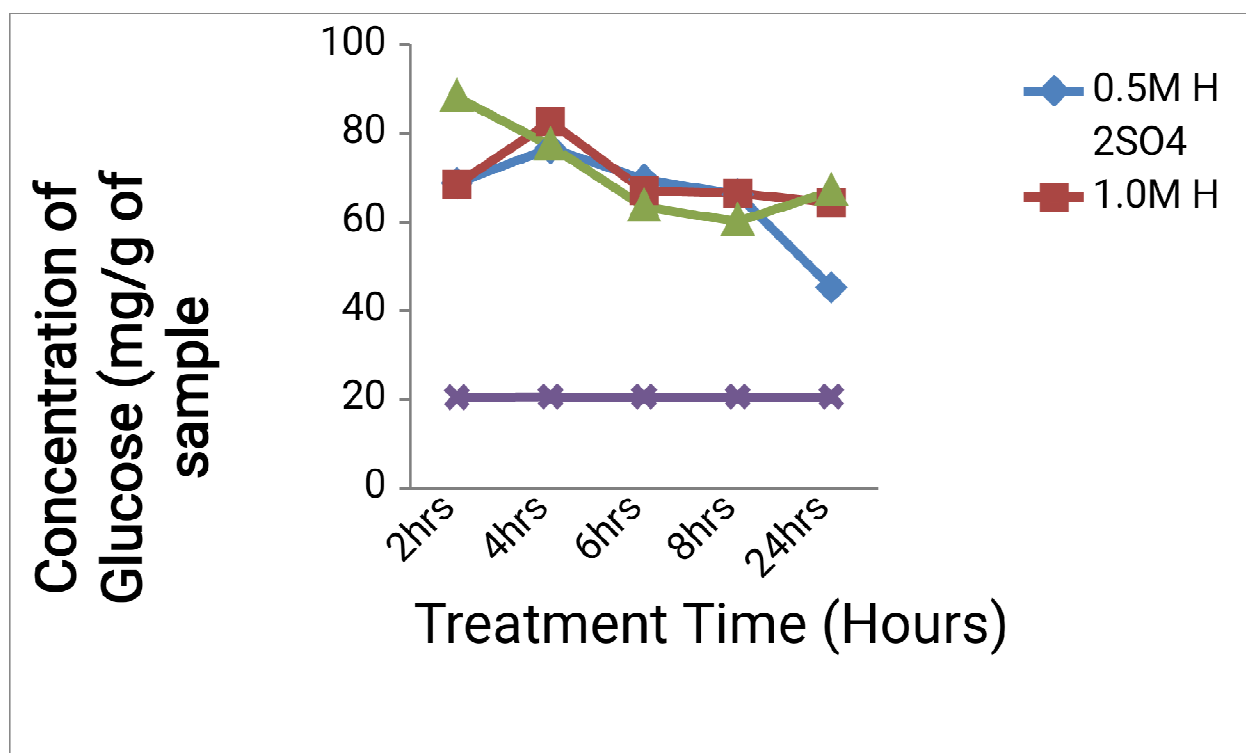


Figure 1: The plot of concentration of glucose against time of treatment with sulfuric acid
 Group 1: Sample + 0.5M H₂SO₄; Group 2: Sample + 1.0M H₂SO₄; Group 3: Sample + 1.5M H₂SO₄; Group 4: Sample + Distilled H₂O.

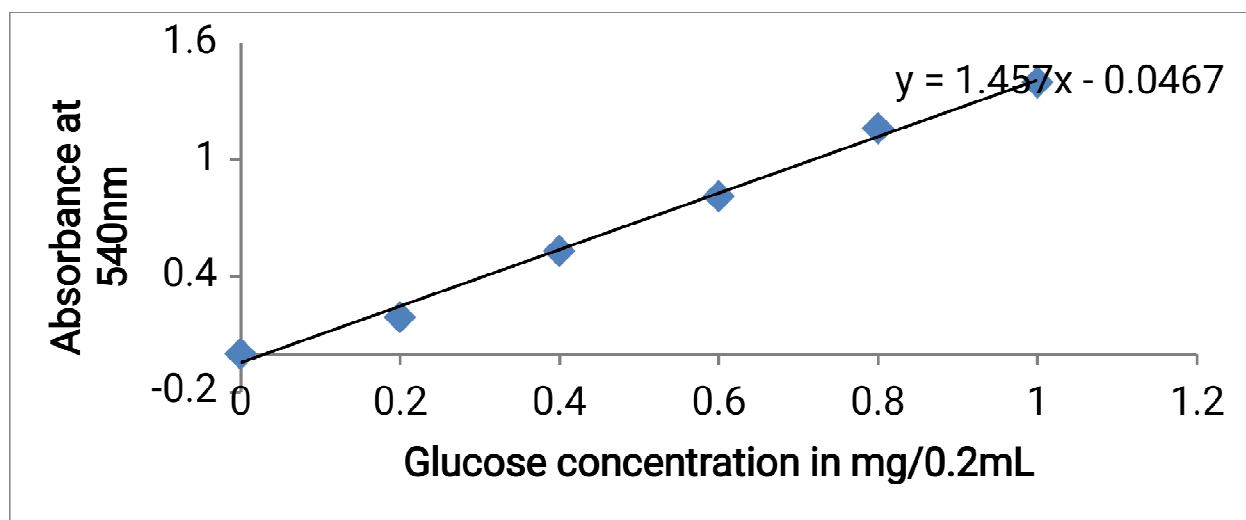


Figure 2: Standard plot of Absorbance against concentration of glucose standards

The plot of the absorbance against the varying concentration of glucose used as standard gave a straight line cutting at the negative absorbance axis (y-axis).

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117 Rice husk contain sugar in the form of polysaccharides such as starch. They need to be pretreated
118 for removal of lignin and disruption of the crystalline structures of cellulose, so that cellulase
119 enzyme can utilize them efficiently. Pretreatment for a long time has been recognized as one of
120 the most expensive processing steps in cellulosic biomass conversion, and several recent articles
121 provide a general overview in the field [17, 18].

122 The results of the present findings demonstrated that 1.5M sulfuric acid induced significant
123 production of monomeric sugars after 2 hours of pretreatment as compared with other
124 concentrations used. However, monomeric sugars production decreases with an increased time of
125 pretreatment, which agrees with reported work on effects of time and acid concentration on the
126 yield of glucose released from solid debris [28]. The reduction in glucose yield over time as
127 shown in the results could be due to the ability of the acid to liberate certain inhibitors (such as
128 fufural, and hydroxyl methylfufural) from the sample during the treatment process, which in turn
129 interferes with the generation of desired fermentable sugar. However, the sugar may be reduce
130 during the washing process to remove the acid prior to enzyme hydrolysis, since the acid has the
131 ability to liberate free sugar from the cellulose chain, thereby leading to the most severe
132 treatments having generated a greater dissolution of sugars in the pretreatment liquid [28].

133 It can be adduced that dilute sulfuric acid is an option for pretreatment of rice husk for the
134 production of fermentable sugar, and thus, agrees with earlier findings of [27, 29] which stated
135 that acid pretreatment of lignocellulose gives high yield of fermentable sugar. The acid has the
136 ability to promotes hydrolysis of hemicelluloses and part of the amorphous cellulose that results

in high recovery of hemicelluloses as monomers in liquid fraction, thus, report on high cellulose content in the solid fraction has been observed [30, 31, 32].

4. Conclusion

The results show that dilute sulfuric acid pretreatment of lignocellulosic materials such as rice husk is an effective method in enhancing the activity of enzymes use in the saccharification of rice husk into fermentable sugar, thereby leading to increased yield. The applications of 1.5M sulfuric acid could maximize the cost effectiveness of the treatment process as seen from the results. Higher concentration may pose more capital cost and may lead to greater loss of the sugar during washing, as a result of the formation of other degradation product which affects enzymatic processes.

References

1. A. Mohammed, S. B. Oyeleke, E. C. Egwim. "Pretreatment and hydrolysis of cassava peels for fermentable sugar". *Asian Journal of Biochemistry*. DOI: 10.3923, 2013.
2. N. R. Baral, J. Li and A. J. Jha. "Fermentable sugars and microbial inhibitors formation from two stage pretreatment of corn stalk with variation in particle size and severity factors". *African Journal of Biotechnology*, vol. 14, no. 39, pp. 2800-2811, 2015.
3. J. Ikwebe and A. P. Harvey. Enzymatic saccharification of cellulose: a study of mixing and agitation in an oscillatory baffled reactor and a stirred tank reactor, *Biofuels*, vol. 6, no. 3-4, pp. 203-208, 2015
4. R. Sims, M. Taylor, J. Saddler, and W. Mabey. "IEA's Report on 1st-to 2nd- Generation Biofuels Technologies" 2009.
5. J. Ikwebe. "Intensification of bioethanol production by simultaneous saccharification and fermentation in an oscillatory baffled reactor." *Thesis submitted to the school of chemical engineering and advanced materials, Newcastle University*. 2012.

6. J. Fischer, V. Lopes, E. F. O. Santos, F. U. Coutinho and V. Cardoso “Second generation ethanol production using crude enzyme complex produced by fungi collected in Brazilian cerrado (Brazilian savanna).” *Chemical Engineering Trans.* vol. 38, pp. 487-492, 2014.
7. S. M. R. Gómez, R. R. Andrade, C. G. Santander, A. C. Costa and F. R. Maciel “Pretreatment of sugar cane bagasse with phosphoric and sulfuric diluted acid for fermentable sugars production by enzymatic hydrolysis.” *Chemical Engineering Trans.* vol. 20, pp. 321-326, 2010.
8. R. B. Gupta and A. Demirbas “Gasoline,diesel and ethanol biofuelss from grasses and plants.” Cambridge: Cambridge University Press, 2010.
9. F. B. Silva, B.B. Romão, V. L. Cardoso, F. U. and Coutinho, E. J. Ribeiro. “Production of ethanol from enzymatically hydrolyzed soybean molasses.” *Biochemical Engineering Journal*, vol. 69, pp. 61-68., 2012.
10. C. Pothiraj, A. Arun and M. Eyini “Simultaneous saccharification and fermentation of cassava waste for ethanol production” *Biofuel Research Journal*, vol. 5, pp. 196-202, 2015.
11. Y. M. Marc. “The potential environmental impact of waste from cellulosic ethanol production.” *Journal of Air & Waste Management. Association*, vol. 60, no.2, pp. 245-250, 2012.
12. H. O. Obueh and M. J. Ikenebomeh. “Bioethanol Production and Compositional Changes during Fermentation of Cassava Processing Wastes from a Local Cassava Mill”, *International Journal Current Research in Bioscience and Plant Biology*, vol. 1, no. 4, pp. 43-51, 2014.
13. A. Silas, M. A. Ospina, S. Delvasto and R. Majia De Gutierrez. “Study on the Pozzolan property of silica obtained from rice husk by chemical and thermal processes.” *IPSS*. vol. 4, no. 11, pp. 4311-4318, 2007.

14. K. Ajay, M. Kalyani, K. Devendra and P. Om. "Properties and industrial applications of rice husk: a review". *International Journal of Emeritus Technology and Advance Engineering*, vol. 2, no.10, pp. 86-91, 2012.
15. O. Mohiuddin, A. Mohiudin, M. Obaidillah, H. Ahmed and S. Asumadu-Sarkodie. "Electricity production potential and social benefit from rice husk, a case study in Pakistan". Doi. 10.1080/23311916.2016.1177156, 2016.
16. S. Asumadu-Sarkodie and P.A. Owusu. "The potential and economic viability of wind farms in Ghana. Energy Resources, Part A: recovery, utilization, and environmental effects." (Taylor and francis online). Doi. 10.1080/15567036.2015.1122680, 2016.
17. P. Alvira, E. Tomás-Pejó, M. Ballesteros, M. J. Negro. "Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review." *Bioresources Technology*. vol. 101, pp. 4851-4861, 2010.
18. F. Carneiro and L. C. Duarte. "Hemicellulose biorefineries: A review on biomass pre-treatments". *Journal of Scientific and Industrial Research*, vol. 67, no. 11, pp. 849-864, 2008.
19. I. C. Nwaneshiudu, I. Ganguly, F. Pierobon, T. Bowers and I. Eastin. (2016). "Environmental assessment of mild bisulfite pretreatment of forest residues into fermentable sugars for biofuels." vol. 9, no. 15, doi 10.1186/s13068-016-0433-1, 2016.
20. N. Mosier, C. Wyman, B. Dale, R. Elander, Y. Lee, M. Holtzapple and M. Ladisch. "Features of promising technologies for pretreatment of lignocellulosic biomass." *Bioresource Technology*. vol. 96, pp. 673-686, 2015.
21. Y. Tyea, K. Leeb, W. Abdullaha, C. Leh. (2012). Potential of Ceiba pentandra (L.) Gaertn (kapok fiber) as a resource for second generation bioethanol: Effect of various simple pretreatment methods on sugar production". *Bioresource.Technology*, vol. 116, pp. 536-539, 2012.

22. I. C. Nwaneshiudu, D. T. Scharzt. “Rational design of polimerised absorbents: application to the fermentation inhibitor fufural.” *Biotechnology Biofuels*,. vol. 8, pp. 72, 2015.
23. J. Gao, D. Anderson and B. Levi. “Saccharification of recalcitrant biomass and integration options for lignocellulosic sugars from catchlight energy process (CLE sugar)”, *Biotechnology Biofuels*.vol. 6, no. 10, 2013.
24. E. P. Dagnino, E. R. Chamorro, S. D. Romano, F. E. Felissia and M. C. Area. “Optimization of the acid pretreatment of rice hulls to obtain fermentable sugars for bioethanol production”. *Journal of Industrial Crops Production*. Vol. 42, pp. 363-368, 2013.
25. H. Fang, C. Zhao and X. Song. “Optimization of enzymatic hydrolysis of steam exploded corn stover by two approaches: response surface methodologyor using cellulase from mixed cultures of *Trichoderma reesei* RUT-C30 and *Aspergillus niger* NL02”. *Bioresource Technology*. 101:4111-4119, 2010.
26. G. L. Milller “Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar”. *Analytical Chemistry*, vol. 31, no. 3, pp. 426-428, 1959.
27. N. Anamaria, P. Viorica, M. M. Manuela, I. Cosmin, A. Beatrice and V. Ostafe V” Adapting the Reducing Sugars Method with Dinitrosalicylic Acid to Microtiter Plates and Microwave Heating” *Journal Brazilian Chemical Society*, vol. 23, no. 12, pp. 2176-2182, 2012.
28. E. P. Dagnino, E. R. Chamorro, S. D. Romano, F. E. Felissia and M. C. Area “Optimization of the acid pretreatment of rice hulls to obtain fermentable sugars for bioethanol production.” *Journal of Industrial Crops Production*. vol. 42, pp. 363-368, 2013.
29. P. F. H. Harmsen, W. J. J. Huijgen, L. M. B. López and R. R. C. Bakker. “Literature review of physical and chemical pretreatment processes for lignocellulosic biomass”. *Energy research centre of the Netherland*, pp.10-013, 2010.

- 240 30. A. Hendriks and G. Zeeman “Review: pretreatments to enhance the digestibility of
241 lignocellulosic”, *Biomass and Bioresource Technology*, vol. 100, pp. 10-18, 2009.
- 242 31. M. Linde M, Jakobsson E, Galbe M, Zachhi G “Steam pretreatment of dilute H₂SO₄-
243 impregnated wheat straw and SSF with low yeast and enzyme loadings for
244 bioethanol production”. *Biomass and Bioenergy*, vol. 32, pp.326-332, 2008.
- 245 32. I. Joseph and H. P. Adam. “Enzymatic saccharification of cellulose: a study of mixing
246 and agitation in an oscillatory baffled reactor and a stirred tank reactor”, *Biofuels*, vol.
247 6, pp. 203-208, 2015.

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