Asian Journal of Environment & Ecology
1(2): 1-6, 2016; Article no.AJEE.30156

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Enzymatic Conversion of Waste (Rice Husk) into Fermentable Sugar through Diluted Acid Pretreatment

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Authors' contributions

This work was carried out in collaboration between all authors. Author OO got the concept, design of the study and its interpretation. Author TOI carried out the laboratory aspect of the work. Author FBO participated in the work acquisition of data and draft of the raw manuscript. Author CCE participated in the laboratory analysis. Author SIRO critically revised the draft manuscript for important intellectual content. All authors read through manuscript and gave a final approval of the revised version to be published.

Article Information

DOI: 10.9734/AJEE/2016/30156
Editor(s):
(1) Anthony R. Lupo, Department of Soil, Environmental, and Atmospheric Science, University of Missouri, Columbia, USA.
Reviewers:
(1) Arun S. Kharat, Dr. Babasaheb Ambedkar Marathwada University, Subcampus Osmanabad, India.
(2) Ify L. Nwaogazie, University of Port Harcourt, Nigeria.
(3) Lincoln Villi Gerke, Universidade Estadual do Oeste do Paraná, Cascavel, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/17706

Original Research Article

Received 21st October 2016
Accepted 15th November 2016
Published 3rd February 2017

ABSTRACT

The enzymatic production of fermentable sugar from rice husk was examined using diluted sulfuric acid as a pretreatment medium. In this study, 10 g of rice husk were separately pretreated with 0.5 M, 1.0 M and 1.5 M sulfuric acid at different time interval of 2-24 hours, prior to saccharification by cellulase enzyme at 37℃ and pH of 4.5. Then the quantity of monosaccharide produced was determined spectrophotometrically. After two hours of pretreatment, the 1.5 M H_2SO_4 pretreated samples yielded 88.05 mg/g (39.11%) of glucose, 1.0 M H_2SO_4 pretreated samples yielded 68.4 mg/g (30.38%), and 0.5M H_2SO_4 pretreated samples yielded 68.7 mg/g (30.51%) respectively, when

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compared with the un-pretreated sample (20.4 mg/g). However, after two hours, the production of glucose significantly reduced with increase time, except with 1.0 and 0.5 M H_2SO_4 pretreated sample which significantly increase at 4 hours (82.5 and 76.5 mg/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.

Keywords: Lignocelluloses; 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 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 environmental 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 paddies are 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 its less commercial interest, or handling and transportation. Therefore, the commercial use of rice husk and its ash is an alternative solution to its disposal problem [14]. Rice husk contains 75-90% organic matter such as cellulose and lignin, while the rest are 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].

2. MATERIALS AND METHODS

2.1 Materials/Chemicals

Cellulase extracted from *Trichodermareesei*, 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 Sample Collection

Four hundred grams (400 g) 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 250 ml conical flasks. To a set of five flasks labeled 2 hr, 4 hr, 6 hr, 8 hr and 24 hr, 90 mL of 0.5 M sulfuric acid was added respectively, to represent group 1. To another set of five flasks labeled 2 hr, 4 hr, 6 hr, 8 hr and 24 hrs, 90 mL of 1.0 M sulfuric acid was added to represent group 2. The third set of five flasks labeled 2 hr, 4 hr, 6 hr, 8 hr and 24 hrs, 90 mL 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 hours, the flasks labeled 2 hr 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 4 hr, 6 hr, 8 hr, and 24 hr labeled preparations. The resulting dried pretreated samples were stored and labeled accordingly [22].

2.4 Enzyme Hydrolysis

Enzymatic hydrolysis was carried out by method described by Fang et al. [23], using commercial cellulase enzyme, purchased from Zayo Sigma [ZSA] Chemical Ltd Denmark.

Two grams of each pretreated sample was weighed inside separate 30 mL test tubes and labeled accordingly, followed by addition of 15mL citrate buffer (pH 4.5) and 200 µL of tetracycline and griseofulvine (prepared by dissolving 500mg of each in a combined 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 of 48 hrs at 37℃ and pH of 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 Miller [24], as modified by Anamaria et al. [25].

3. RESULTS AND DISCUSSION

The plot of concentration of glucose against time of pretreatment is presented in Figs. 1 and 2. In this study, sulfuric acid was 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 68.7 mg/g upon pretreatment with 1.5, 1.0 and 0.5M H_2SO_4 respectively, when compared with the un-pretreated sample (20.4 mg/g). However, after two hours, the production of glucose significantly reduced with increasing time, except with 1.0 and 0.5 M H_2SO_4 pretreated sample which significantly increased at 4 hours (82.5 and 76.5 mg/g respectively).

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

The results of the present findings demonstrated that 1.5M sulfuric acid induced significant production of monomeric sugars after 2 hours of pretreatment as compared with other concentrations used. However, monomeric sugars production decreases with an increased time of pretreatment, which agrees with reported work on effects of time and acid concentration on the yield of glucose released from solid debris [26]. The reduction in glucose yield over time as shown in the results could be due to the ability of the acid to liberate certain inhibitors (such as fufural, and hydroxyl methylfufural) from the sample during the treatment process, which in turn interferes with the generation of desired fermentable sugar. However, the sugar may be reduced during the washing process to remove the acid prior to enzyme hydrolysis, because the acid has the ability to liberate free sugar from the cellulose chain, thereby leading to the most

severe treatments having generated a greater dissolution of sugar in the pretreatment liquid [26].

It can be adduced that dilute sulfuric acid is an option for pretreatment of rice husk for the production of fermentable sugar, and thus, agrees with earlier findings of [25,27] which stated that acid pretreatment of lignocellulose gives high yield of fermentable sugar. The acid has the ability to promote 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 [28,29,30].

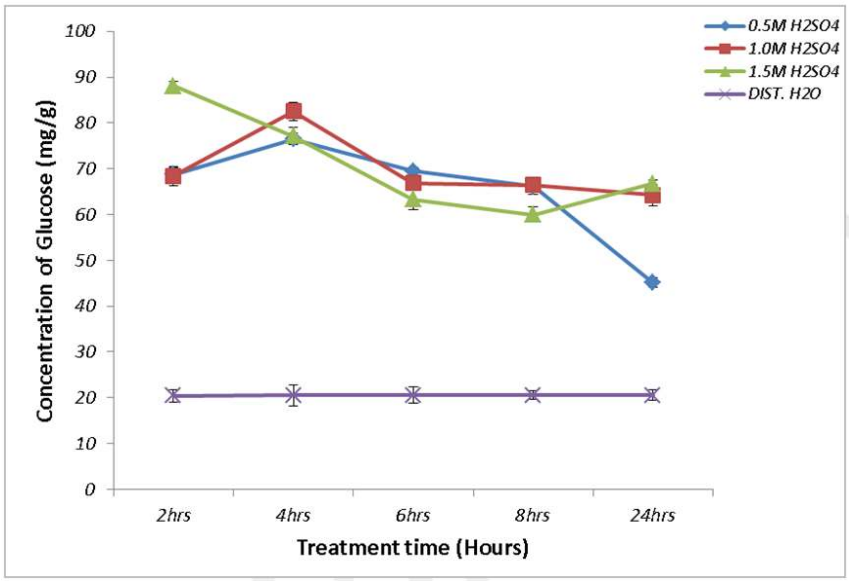

Fig. 1. The plot of concentration of glucose against time of treatment with sulfuric acid
Group 1: Sample + 0.5M H_2SO_4; Group 2: Sample + 1.0M H_2SO_4; Group 3: Sample + 1.5M H_2SO_4; Group 4: Sample + Distilled H_2O

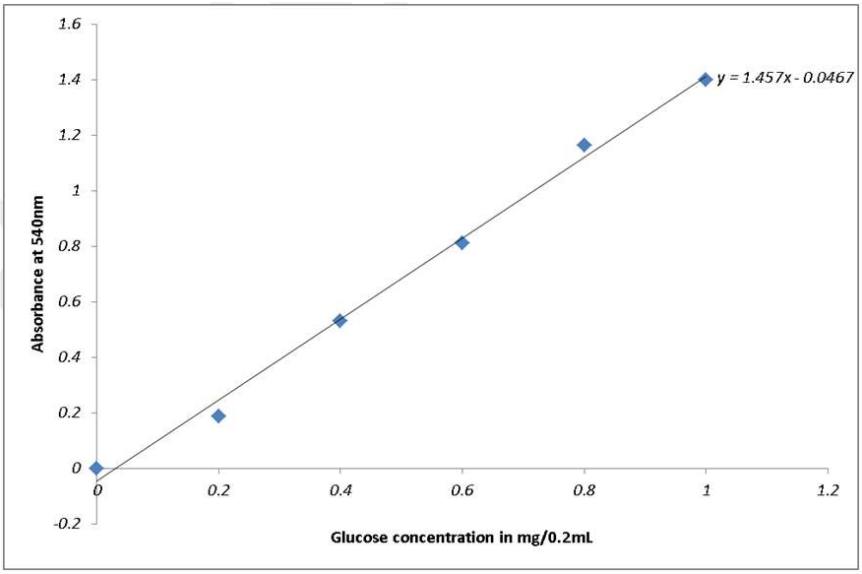

Fig. 2. Standard plot of glucose concentration

4. CONCLUSION

The results showed 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 1.5 M sulfuric acid could maximize the cost effectiveness of the treatment process as seen from the results. Higher concentration may attract more capital cost and may lead to greater loss of the sugar during washing, as a result of the formation of other degradation products which affects enzymatic processes.

ACKNOWLEDGEMENTS

Authors express gratitude to Dr. J. Ikwebe of Biochemistry Department, University of Agriculture, Makurdi-Nigeria, for his technical support in the research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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