International Journal of Basic and Applied Biology
Print ISSN: 2394-5820, Online ISSN: 2349-2539, Volume 2, Number 4; January-March, 2015, pp. 225-227

http://www.krishisanskriti.org/ijbab.html

Saccharification of Wheat Bran and Kitchen Waste for Ethanol Production

Shelja[1] and Rinku Walia[2]

[1,2]Department of Civil Engineering Baddi University of Emerging Sciences & Technology Baddi, Distt. Solan, Himachal Pradesh
E-mail: [1]shelja87@gmail.com, [2]waliarinks@rediffmail.com

Abstract—*Bioconversion of lignocellulose biomass to bioethanol has shown environmental, economic and energetic advantages in comparison to bioethanol produced from sugar or starch. However, the pretreatment process for increasing the enzymatic accessibility and improving the digestibility of cellulose is hindered by many physical-chemical, structural and compositional factors, which make these materials difficult to be used as feedstocks for ethanol production. A wide range of pretreatment methods has been developed to alter or remove structural and compositional impediments to (enzymatic) hydrolysis over the last few decades; however, only a few of them can be used at commercial scale due to economic feasibility. The objective of the present study is to study the pretreatment and enzymatic hydrolysis for bioethanol production using wheat bran and kitchen waste as substrate. Optimization of saccharification conditions was studied at different pH (3,4,5,6,7and 8) and temperature (20,25,30,35,40 and 45^oC) respectively. Optimum conditions of saccharification were achieved at 5pH and 30^oC temperature at 5th day for kitchen waste whereas for wheat bran, it was observed at 4th day.*

Keywords: *Bioethanol, Saccharification, Substrate.*

1. INTRODUCTION

With the growing human population, the world is facing tremendous pressure to meet its needs of food, feed, chemicals, and energy, and also to balance the demand and supply in keeping with environmental safeguards. Today fossil fuels take up 80% of the primary energy consumed in the world, of which 58% alone is consumed by the transport sector (Escobar et al, 2009) and transportation sector accounts for more than 70% of global carbon monooxide (CO) emissions and 19% of global carbon dioxide (CO_2) emissions (Goldemberg et al, 2008). The natural gas and oil fields are shrinking fast to meet the demands of our progress. Thus, the increase concern for the security of oil supply and negative impact of fossil fuel on environment particularly global warming has put pressure on society to find renewable fuel alternative (He et al, 2010).

Bioethanol is one of the promising future energy alternatives contributing to the reduction of negative environmental impacts generated by the use of fossil fuels (McMillan, 1994). Ethanol is environmentally beneficial energy source and can be employed to replace octane enhancers such as methylcyclopentadienyl manganese tricarbonyl (MMT) and aromatic hydrocarbons such as benzene or oxygenates such as methyl tertiary butyl ether (MTBE). For production of biocommodities from biomass one of the major bottlenecks has been the lignocellulosic biomass including environmental wastes and energy crops such as corn, wheat straw, rice husk and sugar cane are high priority research interests worldwide which is .most abundant and low- cost biomass over the world can be used as raw materials for the production of fuel ethanol and develop bioindustries that could support growth of international biofuel market and contribute to the reduction of greenhouse gas emission worldwide (Madhavan et al, 2012).

Lignocelluloses are composed of cellulose, hemicellulose, lignin, extractives, and several inorganic materials. Lignocellulosic conversion mainly includes three processes: pretreatment, hydrolysis of cellulose in the lignocellulosic material to fermentable reducing sugar and fermentation of reducing sugar to ethanol by microorganisms i.e. bacteria, yeast, or filamentous fungi. (Kanafusa-Shinkai et al, 2013). The complexity of production process depends on the feed stock and accordingly spectrum of implemented technology goes from the simple conversion of sugars by fermentation to the multistage conversion of lignocellulosic biomass to ethanol. Pretreatment effectiveness and enzymatic hydrolysis has been correlated with the removal of hemicellulose, lignin and reduction of cellulose fiber crystallinity (Jagtap et al, 2013).

2. MATERIAL AND METHODS

2.1. Materials & microorganism

Wheat Bran and kitchen waste were used as a substrate. The kitchen waste was collected from mess of BUEST, Baddi, Himachal pradesh. It was dried in sunlight and ground to 1mm particle size which is used for further analysis. The fungal spore of *Aspergillus niger* and *Penicillium citrinum* were used for the present study All the experiments were performed in triplicates and the average values were calculated.

2.2. Chemical Pretreatment

2.2.1. Acid treatment In this process the wheat bran and kitchen waste were soaked in 0.5 to 2% of H_2SO_4 for *10* minutes at room temperature and then washed with double distilled water and autoclaved at 121°C with 15 psi pressure for *1* hour (Talebnia et al, 2008).Then the treated substrate was washed with double distilled water until the filtrate becomes neutral. The substrates were dried at 50°C in hot air oven for subsequently analysis.

2.2.2. Alkali treatment Both waste were treat with 0.5 to 2% of NaOH, excess of sulfuric acid was added into each flask to soaked wheat bran and kitchen waste for *10* min at room temperature and then washed with double distilled water and autoclaved at 121^{0}C with 15 psi pressure for *1* hour. Then the treated substrate was washed with double distilled water until the filterate becomes neutral. The substrates were dried at 50^{0}C in hot air oven for subsequently analysis.

2.3. Enzymes Hydrolysis

2.3.1. Inoculum source A pre - isolated and pre – identified strain of *Penicillium citrinum and Aspergillus niger* as used for present study.

2.3.2. Inoculum Preparation A homogeneous spore suspension was obtained by adding one crop of colonies into Erlenmeyer flask's containing broth media and incubating the flasks in incubator at 30 ^{0}C for *5* days till sporulation (Cunha et al, 2012). An appropriate concentration of 1×10^6-- 1×10^7 spores/ml was obtained (Maeda et al, 2013). 1ml of the spore was added to the each of both flasks containing Kitchen waste, Wheat bran substrates and mineral media, under the sterilized conditions.

2.3.3. Standardization of Conditions for Saccharification To determine the optimum saccharification conditions of pH, temperature and time, the above mention reaction mixture was incubated from 3 to 8 pH at 20 to 45^{0}C temperature for *10* days at 120 rpm. These are the most important parameters affecting the growth of fungus.

The reducing sugar was analyzed by DNS method for optimum conditions.

The percentage saccharification was calculated as (Baig et al, 2004)

$$\text{Saccharification (\%)} = \frac{\text{Glucose (mg/ml)} \times 100}{\text{Substrate (mg/ml)}}$$

3. RESULTS AND DISCUSSION

3.1. Chemical pretreatment of kitchen waste

3.1.1. Effect of sulfuric acid and alkali pretreatment The effect of acidic and alkali pretreatment on lignin, hemicelluloses and cellulose was investigated by conducting experiments at different concentrations of sulfuric acid and sodium hydroxide from 0.5 to 2.0% for both the waste. The effect of sulfuric acid pretreatment on lignin, hemicellulose and cellulose removal was investigated. Pretreatment with acid predominantly increases the surface area of lignocellulosic material, making the polysaccharides more susceptible to enzymatic hydrolysis (Zheng et al, 2014). The main types of bonds that connect the building molecules within the lignin polymer are ether bonds and carbon-to-carbon bonds. The use of an alkali causes the degradation of ester and glycosidic side chains resulting in structural alteration of lignin, cellulose swelling, partial decrystallization of cellulose (Cheng et al, 2010; McIntosh and Vancov, 2010 and Ibrahim et al, 2011) and partial solvation of hemicelluloses (Sills and Gossett, 2011). Sodium hydroxide has been extensively studied for many years, and it has been shown to disrupt the lignin structure of the biomass, increasing the accessibility of enzymes to cellulose and hemicelluloses (Zhao et al, 2009).

3.2. Optimization of Culture Conditions for Enzyme Production

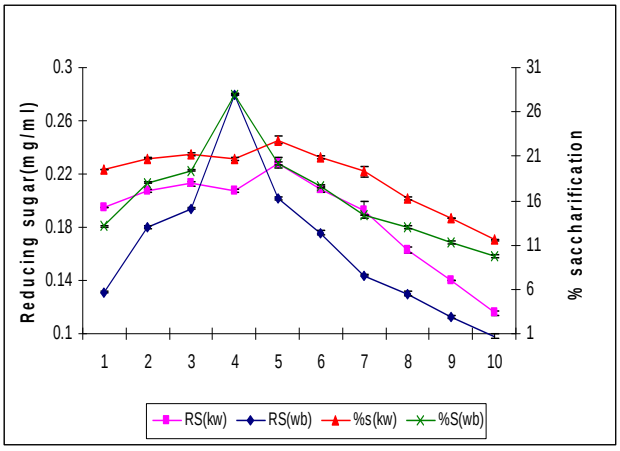

Fig. 1: Effect of contact time (Days) on saccharification of wheat bran and kitchen waste

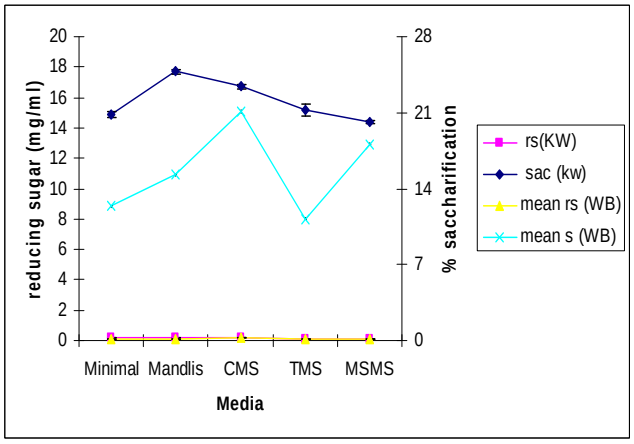

Fig. 2: Effect of media on saccharification of wheat bran and kitchen waste

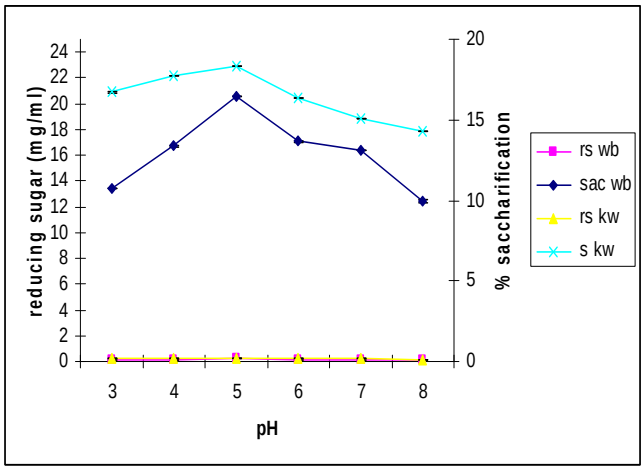

Fig. 3: Effect of pH on saccharification of wheat bran and kitchen waste

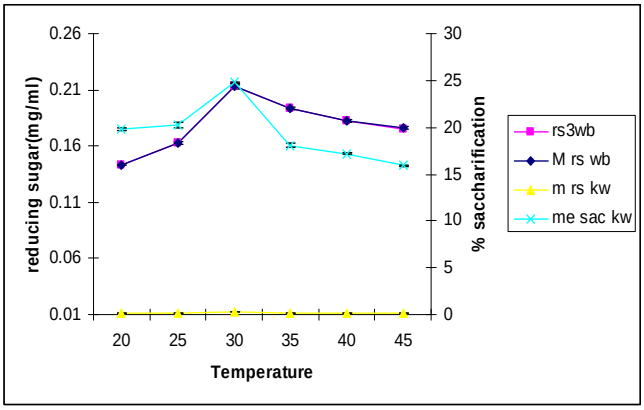

Fig. 4: Effect of temperature on saccharification of wheat bran and kitchen waste

4. CONCLUSION

The investigation of pretreatment and hydrolysis of kitchen waste and wheat bran for bioethanol production was studied by *P. citrinum* and *A. niger*. The main purpose of pretreatment is to remove hemicelluloses and lignin, to increase the accessible surface area for enzymes and to descrystallize cellulose. Both kitchen waste and Wheat bran is cheap residue which can be used as a substrate for enzyme production which reduces the cost of enzyme production and enzymatic conversion of carbohydrate part of both wastes into fermentable sugar (Karunanithy et al., 2013).

REFERENCES

[1] Baig, M.M.V.; Baig, M.L.B.; Baig, M.I.A. and Yasmeen, M., "Saccharification of banana agro-waste by cellulolytic enzymes", *Afr. J. Microbiol. Biotechnol*, 3, 9, 2004, pp. 447-450.

[2] Cheng, Y. S., Zheng, Y., Yu, C. W., X Dooley, C. W.,. Jenkins, B. M and J. S. VanderGheynst., "Evaluation of high solids alkaline pretreatment of rice straw", *Appl. Biochem. Biotechnol.* 162, 6, 2010, pp. 1768–1784.

[3] Cunha F.M., Esperanca M.N., Zangirolami T.C., Badino, A.C., Farinas, C.S., "Sequential solid-state and submerged cultivation of *Aspergillus niger* on sugarcane bagasse for the production of cellulose", *Bioresour Technol*, 112, 2012, pp.270–274.

[4] Escobar, J.C., Lora, E.S., Venturini, O.J., Yanez, E.E., Castillo, E.F. and Almazan, O., "Biofuels: environment technology and food security" *Renew. Sustain. Eng.*, 13, 7, 2009, pp. 1275-87.

[5] Goldemberg, J., "Environmental and ecological dimensions of biofuels" In proceeding of the conference on ecological dimensions of biofuels, Washington, D.C., 2008.

[6] He, Y.; Wang, S. and Lai, K.K., "Globaleconomic activity and crude oil price: a cointegration analysis", *Energy*, 63, 2010, pp. 12-17.

[7] Ibrahim, M. M., El-Zawawy, W. K., Abdel-Fattah, Y. R., Soliman, N. A. and Agblevor, F.A., "Comparison of alkaline pulping with steam explosion for glucose production from rice straw", *Carbohydrate Polymers*, 83, 2, 2011,pp. 720–726.

[8] Jagtap, S.S., Dhiman, S.S., Kim, T.S., Li, J., Lee, J.K., Kang, Y.C., "Enzymatic hydrolysis of aspen biomass into fermentable sugars by using lignocellulases from *Armillaria gemina*", *Bioresour Technol*, 133, 2013, pp. 307–314.

[9] Kanafusa-Shinkai, S., Wakayama, J., Tsukamoto, K., Hayashi, N., Miyazaki, Y., Ohmori, H., Tajima, K., Yokoyama, H., "Degradation of microcrystalline cellulose and non-pretreated plant biomass by a cell-free extracellular cellulase/hemicellulase system from the extreme thermophilic bacterium", *J Biosci Bioeng*, 115, 2013, pp.64–70.

[10] Karunanithy, C.; Muthukumarappan, K.; Gibbons, W.R., "Effect of extruder screw speed, temperature, and enzyme levels on sugar recovery from different biomasses", *ISRN Biotechnol.* 2013, pp. 1-13.

[11] Madhavan A, Srivastava A, Kondo A, Bisaria VS. "Bioconversion of lignocellulosederived sugars to ethanol by engineered *Saccharomyces cerevisiae*", *Crit Rev Biotechnol*, 32, 2012, pp. 22–48.

[12] Maeda, R.N., Barcelos, C.A, Anna, L.M.M.S., Pereira, N. Jr.,"Cellulase production by *Penicillium funiculosum* and its application in the hydrolysis of sugar cane bagasse for second generation ethanol production by fed batch operation" *J Biotechnol*, 163, 2013, pp.38–44.

[13] Mc Intosh, S. and Vancov, T., (2010) "Enhanced enzyme saccharification of Sorghum bicolor straw using dilute alkali pretreatment", *Biores. Technol*, 101, 17, 2010, pp. 6718–6727.

[14] Millan, M.C.D., "Pretreatment of lignocellulosic biomass" 1994.

[15] Sills, D. L. and Gossett, J.M., "Assessment of commercial hemicellulases for saccharification of alkaline pretreated perennial biomass", *Biores. Technol.*102, 2, 2011, pp. 1389–1398.

[16] Talebnia, F., Bafrani, M.P., Lundin, M. and Taherzadeh, M.J., "Optimization study of citrus wastes saccharification by dilute acid hydrolysis", *Biores.* 3, 2008, pp. 108-122.

[17] Zhao, H., Jones, C. L., Baker, G. A., Xia, S., Olubajo, O. and Person, V.N., "Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis" *J. Biotechnol.* 139, 1, 2009, pp. 47–54.

[18] Zheng, J., Choo, K., Bradt, C., Lehoux, R., Rehmann, L., "Enzymatic hydrolysis of steam exploded corncob residues after pretreatment in a twin-screw extruder", *Biotechnol. Rep. 3*, 2014, pp. 99–107.