Vol. 12 (1) 103-107 January 2018, ISSN 0973-8916 (Print), 2230-7303 (Online)

Effect of Metal ions on the Dilute Acid Pretreatment of Lignocellulosic Biomass

Abdul Majid Khan*[1], Sanjay Sahay[2], Kirti Jain[1] and Ragini Gothalwal[3]
[1]Department of Botany, Government Science and Commerce College, Benazeer, Bhopal (MP) India.
[2]Department of Botany, Government Postgraduate College, Biaora, Dist. Rajgarh (MP) India.
[3]Department of biotechnology and bioinformatics centre, UTD, Barkatullah University, Bhopal
*For Correspondence - mamk_khan300@yahoo.com

Abstract

Economically feasible, sustainable and non-polluting ways for ethanol production has become important due to limited petroleum supply and associated environmental consequences of burning fossil fuel. Three experimental lignocellulosic materials viz., *Hyptis suaveolens*, wheat straw and *Ailanthus exelsa* wood were taken to subject them to dilute acid pretreatment in presence of selected metals. Metals Zn and Ca were found general inhibitors of release of hemicellulosic sugars. *A. exelsa* wood was found to be more sensitive to metals in the release of hemicellulosic sugars during pretreatment by dilute acid method. Metals were also found to interfere with the utilization of sugar and ethanol fermentation by the yeast.

Key words: Pretreatment, metal ion effect, lignocelllulose.

Introduction

Fossils are supposed to have been produced by decomposition of dead plants and animals in millions of years, so fossil fuel is limited and non-renewable per se. Fossil fuels are organic hydrocarbons that on burning gives off carbon dioxide, a known greenhouse gas largely responsible for the climate change. In view of this, there is increased demand and interest in alternative fuel especially liquid transportation fuel all over the world (1). Transport fuel from biomass called biofuel thus emerged as the most important alternative fuel.

Of various biofuels, bioethanol as petroleum extender in the existing vehicles has been in use since 1970s and is expected to be used as singular fuel in future vehicles with suitably modified engine. Initial bioethanol called 1G bioethanol is obtained from starch crops such as corn and sugar cane. Since, IG ethanol has been blamed for its role in food verses fuel competition, attention has now been focused on 2G bioethanol whose feedstock is waste lignocellulosic materials (2).

Lignocellulosic biomass as feedstock is abundant and renewable and thus the product bioethanol is considered as the sustainable energy resources (3,4). Lignocellulose has fermentable glucose in polymerized form as cellulose which in turn is intertwined with lignin and hemicelluloses. In order to expose cellulose to enzyme or alternative physical method to release glucose from it, pretreatment of lignocellulosic is necessary. Of various pretreatment methods (5), dilute acid one of the most widely accepted method (6). The method applies 0.4-1% sulphuric acid to lignocellulosic material, the process often called impregnation followed by heat treatment (7). The reaction thus involves substrate lignocellulosics and three reagents viz., heat, acid and water. Of these three reagents the quality of former two is largely under control, the quality of natural supply of water however is extremely variable especially in terms of dissolved metals. At commercial level it is obvious that a very high quality of water may not be used everywhere and thus data about the role of various metal ions present in natural water in the efficiency of dilute acid pretreatment method must be available.

In the present study therefore the effect of some commonly occurring metals in natural water on the release of hemicellulose and lignin degradation products during dilute acid pretreatment from lignocellulosics was tested.

Materials and Methods

Plant materials : *Hyptis suaveolens* a wild weed found abundantly in north India, *Ailenthus exelsa* a potent energy plant (7,8) and a highly appreciated agro-waste viz., wheat straw were used as feed-stocks. The plant biomass of all the three materials was milled with particle size about 1mm x 0.5cm x 0.5cm, air dried to 10% moisture and stored at room condition until use.

Impregnation of biomass : Before the hydrolysis step, 100 gm of biomass from each source was impregnated with 0.7% v/v H_2SO_4 with 1mM of metal ions like Zn ($ZnCl_2$), Ca ($CaCl_2$), Fe ($FeCl_2$), Cu ($CuSO_4$) and Mg ($MgSO_4$) to a final volume of 1000 ml in separate flasks and incubated overnight. After the incubation, the excess fluid was removed. The treated woodchips were cooked at 121^{O}C for 30 min in autoclave. The wood prehydrolysate obtained by above process was recovered by filtration with Whatman No.4.

Treatment of hydrolysate :The hemicellulose acid prehydrolysate (HAP), was once more heated to 100^{o}C for 15 min to break down oligosaccharide into monomers of hexoses and pentoses. The hydrolysate was basified with solid NaOH (9) or over limed with limestone (10) until the pH reached 9.0 - 9.5. Insoluble residues from treated HAP were removed by filtration (Whatman No.4), and the supernatant was collected for further use as fermentable sugars (9). Triplicate samples of the hydrolysate were analyzed to estimate the amount of fermentable sugars.

Microorganism and its maintenance : *S. stipitis* (formerly *P. stipitis*) NCIM 3507 was obtained from National Collection of Industrial Microorganism (NCIM), NCL, Pune. It was maintained on agar slants containing (g l^{-1}): xylose, 20; yeast extract, 3; malt extract, 3; peptone, 5; and agar, 20. The medium used for inoculum preparation contained (g l^{-1}): D-xylose, 50; glucose, 5; yeast extract, 3; malt extract, 3; peptone 5; pH 5. To prepare the inoculum, a 250-ml Erlenmeyer flask containing 50 ml medium was inoculated with microbe from a fresh agar slant, and incubated at 30°C on a rotary shaker at 150 rpm for 48 h prior to use. A 5 ml aliquot of this culture was transferred to 100 ml fermentation medium.

Fermentation : Anaerobic batch fermentation of 100 ml broth consisting of 70% pretreated, detoxified hydrolysate of *H. suaveolens*, wheat straw and *A. excelsa* and 30% supplementary medium in separate conical flasks was carried out. Supplementary medium consisted of (g/l) glucose, 20; yeast extract, 3; peptone, 5; KH_2PO_4, 2; $(NH_4)_2SO_4$, 1; $MgSO_4.7H_2O$, 0·5; trace element solution, 1 ml l^{-1}; the pH adjusted to 5·0 ± 0·1. The trace element solution contained (g l^{-1}): $CuSO_4.5H_2O$, 2·5; $FeCl_3.6H_2O$, 2·7; $MnSO_4.H_2O$, 1·69; $Na_2MoO_4.2H_2O$, 2·42; $ZnSO_4.7H_2O$, 2·87; $CoCl_2.6H_2O$, 2·38; and H_2SO_4 (conc.) 3 drops (11). Complete sterilization was done by autoclaving at 121^{o} C, 15psi for 30 min. Fermentation was carried out at temperature 32^{o}C with agitation at 100 rpm on shaker incubator for six days.

Analytical methods : Assay of total reducing sugar was carried out by DNS (dinitrosalycilate) method (12) pentose sugar by Orcinol reagent method (13). Ethanol was quantified in the distillate applying Potassium dichromate reagent method (14).

Statistical control : All experiments were performed in triplicates and three times. Statistical significance of the means was evaluated using one-way analysis of variance. Subsequent comparisons were performed using the least significant difference (LSD) test. Differences were considered significant when $P < 0.05$.

Results and Discussion:

Effect of metal ions on H. suaveolens wood pretreatment : As a result of pretreatment in presence of various metals, maximum negative impact was found with Zn that reduced release of sugars to 30% level of that obtained without any metal. On the other hand least impact was found with Mg. All other metals showed negative impacts although their magnitude differed (Fig. 1). The release of phenolics was hardly influenced by the presence of tested metals.

Effect of metal ions on wheat straw pretreatment : In case of pretreatment of wheat straw in presence of various metals, maximum negative impact was found with Ca that reduced release of sugars to 20% level. On the other hand least impact was found with Mg. All other metals showed negative impacts although their magnitude differ (Fig. 2). In case of phenolics, the release was rather stimulated by Fe, Mg and Cu.

Effect of metal ions on A. exelsa wood pretreatment : In case of *A. exelsa* wood the result of pretreatment in presence of various metals showed maximum negative impact with Fe that reduced release of sugars to 20% level. On the other hand least impact was found with Mg. All other metals showed negative impacts with their magnitude varied between 70-80 % levels (Fig. 3). In case of phenolics, the release was influenced by the tested metals.

From the above results it can be deduced that Mg hardly had any impact on the release of sugars or phenolics due to pretreatment of plant biomass. It seems that Mg has nothing to do with the structural integrity of the wood. On the other hand Zn and Ca seem to play some role in the structural configuration of the wood especially herb and shrub. In case of *A. exelsa* wood, apart from Zn and Ca, Fe was also found to have considerable impact on the release of sugars and thus all the three seem to play some role in the structural configuration of the tree wood.

Dilute acid pretreatment method was found suitable and economic earlier (15,16) and 0.7% acid was found to be optimum for pretreatment of *A. exelsa* wood (7,8).

***Effect of metal ions on the fermentation of treated H. suaveolens* wood hydrolysate :** The hemicelluloses hydrolysate from *H. suaveolens* wood was treated with lime and subjected to

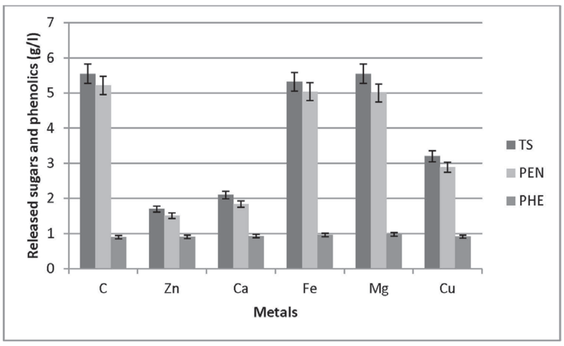

Fig. 1 Concentration of sugars and phenolics in the hydrolysate of *Hyptis suaveolens* wood released in presence of various tested metals

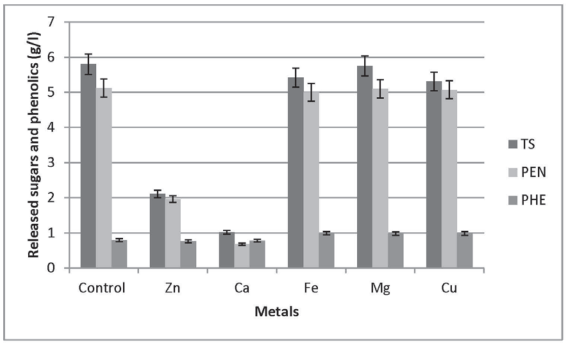

Fig. 2 Concentration of sugars and phenolics in the hydrolysate of wheat straw released in presence of various tested metals

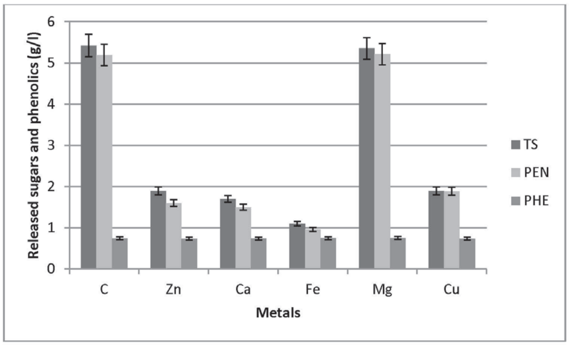

Fig. 3 Concentration of sugars and phenolics in the hydrolysate of *Ailanthus exelsa* wood released in presence of various tested metals (TS-total sugar; PEN-pentoses; PHE-phenolics)

fermentation. There was a little impact on the utilization of sugars and production of ethanol (Fig. 4).

Effect of metal ions on the fermentation of treated wheat straw hydrolysate : The hemicelluloses hydrolysate from wheat straw was treated with lime and subjected to fermentation. Although there was little impact, negative effect of Cu and Fe on sugar utilization was evident, on the other hand Mg improved a little bit ethanol fermentation (Fig. 5).

Effect of metal ions on the fermentation of treated A. exelsa wood hydrolysate : The hemicelluloses hydrolysate from *A. exelsa* was treated with lime and subjected to fermentation. There was significant negative impact of all the metals on the sugar utilization by the yeast, ethanol fermentation was also inhibited a little bit by Cu (Fig. 6).

Metals present in the natural water were found in general to inhibit the release of hemicellulosic sugars and lignin. Metal ions were also found to interfere with sugar utilization and ethanol fermentation by the yeast. More specifically, Ca and Zn reduced the release of sugars in case of *H. suaveolens* wood by 62% and 70% respectively and in wheat straw by 82% and 64% respectively. In the case of *A. excelsa* wood, Ca, Zn, Fe and Cu prevented release of sugars by 68%, 63%, 80% and 63% respectively. The different response of woods from different source materials in presence of various metals indicates the compositional or configurational difference of woods. Ca and Zn seem to be general inhibitors of release of sugars during pretreatment by dilute acid method possibly through strengthening the configuration of the wood. Metals were also found to interfere with the fermentation of treated hydrolysate sugars. The result is important and highlights the importance of purity of water to achieve maximum efficiency pretreatment applying dilute acid method.

Conclusions

Ca and Zn are general inhibitors of the

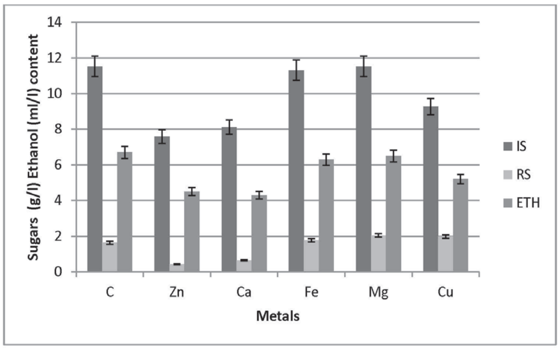

Fig. 4 Sugar utilization from hydrolysate obtained in presence of various metals and its subsequent fermentation to ethanol (IS- initial sugars before fermentation; RS-residual sugars after fermentation; ETH- ethanol)

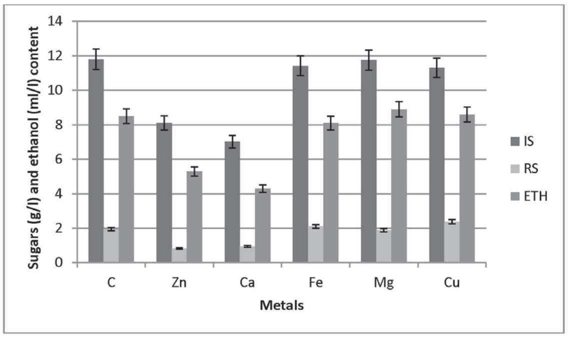

Fig. 5 Sugar utilization from hydrolysate obtained in presence of various metals and its subsequent fermentation to ethanol (IS- initial sugars before fermentation; RS-residual sugars after fermentation; ETH- ethanol)

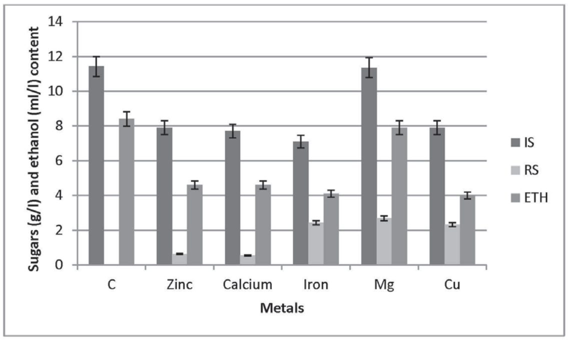

Fig. 6 Sugar utilization from hydrolysate obtained in presence of various metals and its subsequent fermentation to ethanol (IS- initial sugars before fermentation; RS-residual sugars after fermentation; ETH- ethanol)

release of hemicellulosic sugars. Mg has no effect. In case of tree wood Fe and Cu may also inhibit release of hemicellulosic sugars. It seems that structural integrity of various types of plants (herb, shrub and tree) depends on some metals as well. More works involving more types of plants are required to get a fuller appreciation of general role of metals on the efficiency of pretreatment of wood applying dilute acid or other methods.

References

1. Wyman, C.E. (2007). What is (and is not) vital to advancing cellulosic ethanol. Trends Biotechnology. 25(4):153-157.

2. Taherzadeh, M.J. and Karimi, K. (2007). Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A review. BioResource 2, 472-499.

3. Grad, P. (2006). Biofuelling Brazil: An Overview of the Bioethanol Success Story in Brazil. Refocus3, 56-59.

4. Sukumaran, R.K., Surender, V. J., Sindhu, R., Binod, P., Janu, K. U., Sajna, K. V., Rajasree, K. P., Pandey, A. (2009). Lignocellulosic ethanol in India: Prospects, challenges and feedstock availability. Bioresource Technology 4826-4833

5. Yang, B. and Wyman, C.E. (2008). Pretreatment: the key to unlocking low-cost cellulosic ethanol. Biofuels, Bioproducts Biorefining. 2:26–40.

6. Chandel, A.K., Singh, O.V., Rao, L.V. (2009). Biotechnological Applications of Hemicellulosic Derived Sugars: State-of-the-Art. Sustainable Biotechnology. 63-81

7. Jain, P., Rana, R.S., Sahay, S. (2013). Evaluation of a minimal energy-dependent technique for preparation of *Ailanthus excelsa* hemicellulose acid hydrolysate fermentable by *Schefferomyces stipitis*. Journal of Biofuels 3: 103–111.

8. Sahay, S. and Rana, R.S. (2017). Hemicellulose hydrolysate from *Ailanthus excelsa* wood potentially fermentable to ethanol. Journal of Tropical Forest Science 29(2) 172-178.

9. Tanawut, T., Thikamporn, K., Ponpitak, P., Virapong, P., (2007). Appropriate Technology for the Bioconversion of Water Hyacinth (*Eichhornia crassipes*) to Liquid Ethanol: Future Prospects for Community Strengthening and Sustainable Development EXCLI Journal. 6:167-176.

10. Martinez, A., Rodriguez, M.E., York, S.W., Preston, J.F and Ingram, L.O. (2000). Effects of $Ca(OH)_2$ treatments (“overliming’’) on the composition and toxicity of bagasse hemicellulose hydrolysates. Biotechnology Bioengineering . 69:526-536.

11. Miller, G.L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry. 31, 426-428.

12. Plummer, D.T. (1987). Introduction to Practical Biochemistry (3rd edition), McGraw-Hill Publishing Co., NewDelhi, India, pp 157.

13. Caputi, J.A., Veda, M., Brom, T. (1968). Spectrophotometric determination of ethanol in wine. American Journal of Enology and Viticulture. 19:160-165.

14. Sun, Y. and Cheng, J.J. (2005). Dilute acid pretreatment of rye straw and Bermuda grass for ethanol production. Bioresource Technology 96: 1599–1606.

15. Zhu, S., Wu, Y., Yu, Z., (2006). Microwave-assisted alkali pre-treatment of wheat straw and its enzymatic hydrolysis. Biosystems Engineering 94: 437–442.