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National Academy of Agricultural Science (NAAS) Rating : 3. 03

Acid Pretreatment of Wheat Straw for Bioethanol Production

Seema Dahiya*, Monika Barkodia*, Sneh Goyal* and Leela Wati*

ABSTRACT: *Lignocellulosic substrate can be used for bioethanol production. Wheat straw among lignocellulosic substrate can be used for bioethanol production by using 3 steps: pretreatment, hydrolysis and fermentation. The aim of this study is to found out best acid pretreatment method to make wheat straw a suitable substrate for bioethanol production. For the pretreatment of wheat straw, it was grounded to 1mm size and treated with three concentrations of H_2SO_4 i.e.1, 2 and 3% along with tween 1% tween 20. Wheat straw contained 23.2% cellulose, 30% hemicellulose and 14.1% lignin. After acid pretreatment hemicellulose solublization takes place with the increase in cellulose content and decrease in lignin content. Maximum 55.5% increase in cellulose content along with significant decrease in ligin content was observed in case of finely grounded wheat straw (1mm size) treated with 3% H_2SO_4 along with 1% tween 20 .*

Keywords: *Acid pretreatment, Bioethanol, Wheat straw, Lignocellulose*

INTRODUCTION

Increase in the oil consumptions and environmental problems to 400% in the last decade (Pena *et al.,* 2012). Ethanol production by fermentation is being evaluated in many countries including India as a liquid fuel for automobiles and as a chemical feed stock. Gasoline with upto 10% ethanol can be used in most vehicles without modification. The government of India has also declared the use of 5 % ethanol blend in petrol mandatory in 9 states and 4 union territories. Bioethanol has advantage over petrol as fuel as it is made from biomass and is renewable. As the biomass grows it consumes as much carbon dioxide as it forms during the combustion of bioethanol, which makes the net contribution to the green house effect zero. Currently, bio-ethanol is obtained from the alcoholic fermentation of monosaccharides derived from sugary and starchy crops. As a feedstock for bioethanol, agricultural lignocellulosic biomass such as wheat straw, rice straw and bagasse may be used in the second generation context, as these are abundant around the world (Volynets and Dahman, 2012). India is a country with a positive outlook towards renewable energy technologies and committed to the use of renewable sources to supplement its energy requirements. India has 0.5% of the oil and gas resources of the world but 16% of the world's population thereby depending upon lignocellulosic materials for production of bio energy. Wheat is one of the most important crops in India and its agricultural byproduct, wheat straw, is increasingly being considered as feedstock for the production of fuels, energy and chemicals (Marinkovic *et al.,* 2012). For every 1.3 kg of wheat grain produced, about 1kg of straw is generated, resulted in about 534.23 million tones of wheat straw (Ruiz *et al.,* 2012). Wheat straw offers great potential as an alternate substrate as more than half of its dry weight consists of cellulose and hemicellulose which can be used as a substrate for ethanol production. It contain 35-45%cellulose, 20-30% hemicellulose and 8-15% lignin (Gubitz *et al.,*1998; Montane *et al.,*1998).

To use wheat straw as a carbon-neutral energy source, the pretreatment is a necessary step for utilization of lignocellulosic materials to obtain ultimately high degree of fermentable sugars. It affects the structure of biomass by solubilizing hemicellulose, reducing crystallinity and increasing the available surface area and pore volume of the substrate. However, no perfect pretreatment method has been discovered now because there are variations in terms of suitability of one method for various materials. Physical and physiochemical methods are not so much efficient for maximum lignin removal.

* Department of Microbiology,CCS HAU HISAR-125004, India

Biological methods are much expensive than other methods so cannot used widely to explore bioethanol from lignocellluosic subastances. Among chemical methods acid or alkali pretreatments are mostly used for removal of lignin from lignocellulosic materials (Mosier *et al.* 2005). Therefore, aim of this study is to find out an effective acid pretreatment method for the production of bioethanol from wheat straw.

MATERIAL AND METHODS

Material Preparation

Wheat straw was obtained from Directorate of Farm, CCS HAU, Hisar. It was dried at 80-90°C and ground to the size of about 1mm.

Pretreatment of Wheat Straw

For pretreatment of wheat straw (1mm), Tween 20-assisted dilute acid pretreatments were optimized at varied sulfuric acid concentration (1–5%, w/v) along with Tween 20 (1% w/v) at a solid to liquid ratio of 1:10 in conical flasks, using autoclave at 121°C for 90 min. Pretreated slurry was filtered through double-layered filtration cloth, washed with distilled water till its pH became neutral and then dried at 70°C for further use.

Analysis of Wheat Straw

Cellulose, hemicellulose and lignin were estimated by determining acid detergent fiber (ADF) and neutral detergent fiber (NDF) in the wheat straw sample (AOAC, 1970).

Reagents ADF solution (g/l)

Component	*Quantity (g/l)*
Cetyltrimethyl ammonium bromide (CTAB)	20.0 g
Sulfuric acid	27.7 ml

Final volume made to one litre with distilled water.

For determination of ADF content, 1 g dried wheat straw was mixed with 100 ml of ADF solution. The contents were refluxed for 1 h on hot plate and filtered through a G1 sintered glass crucible. The residue was washed with hot distilled water till frothing disappeared and finally with acetone. It was dried at 80°C for 24 h, cooled in a dessicator and weighed.

After determination of ADF content, the material in the crucibles were dipped in 72% sulfuric acid and stirred with a glass rod to a smooth paste, breaking all lumps. The crucibles were again half filled with 72% sulfuric acid, stirred regularly at an interval of 1 h and kept for 3 h at 28±2°C. The excess of sulfuric acid was filtered off under vacuum and contents were washed with hot water until free from acid. The glass rods were also rinsed with hot water. The crucibles with material were kept in oven for overnight, cooled in dessicator and weighed again. The loss in weight of the crucibles was expressed as cellulose content. The ashing of the material was done by transferring to a muffle furnace which was ignited at 550°C for 3 h. The crucibles were cooled in dessicator and weighed. The loss in weight of the crucibles was taken as lignin content. **NDF solution (g/l)**

Component	*Quantity (g/l)*
Ethylenediaminetetraacetic acid (EDTA) Disodium salt	18.61
Sodium borate decahydrate	6.81
Sodium lauryl sulphate	30.00
Anhydrous disodium hydrogen phosphate	4.56
2-Ethoxyethanol	10.00 ml

Final volume was made to one litre with distilled water.

For determination of NDF content, 1 g dried wheat straw was mixed with 0.5 g sodium sulfite and 100 ml of NDF solution. The contents were refluxed for 1 h on hot plate and filtered through a G1 sintered glass crucible. The residue was washed with hot distilled water till frothing disappeared and finally with acetone. It was dried at 80°C for 24 h, cooled in a dessicator and weighed.

Hemicellulose content was determined by following formula:

Hemicellulose % = NDF (%) – ADF (%)

RESULTS AND DISCUSSION

Analysis of Wheat Straw

Wheat straw is an agricultural residue after extraction of grains from wheat crop. In India, wheat straw is available in plenty and has an immense potential as a renewable source of energy. The present investigation was aimed at production of bioethanol from wheat straw by simultaneous saccharification and fermentation.

Wheat straw was obtained from Directorate of Farm, CCS HAU, Hisar (Fig. 1). It was dried and powdered to the size of about 1mm (Fig. 1 and 2) and analyzed for various components. The initial analysis of untreated wheat straw is present in table 1 that indicates the presence of 23.2% (w/w) cellulose, 30.0% (w/w) hemicellulose and 14.1% (w/w) lignin. Same type of finding was also reported by various workers. Kuhad and Singh (1993) and Sun and Cheng (2002) reported that cellulose, hemicelluloses and lignin in wheat straw varied from 33 to 40%, 20 to 25% and 15 to 20% respectively.

Taherzadeh and Karimi, 2008 found that milling does not affect the chemical composition of material, but increases the accessible surface and the size of pores of cellulose and decreases the size of chains of carbohydrates and lignin. The requirement of pretreating wheat straw before hydrolysis and fermentation lies in the fact that the pretreatment disrupts the lignin seal and crystallinity of native cellulose making it more accessible to hydrolysis. Kumar *et al.,* (2009) advocated that milling must be combined with some chemical pretreatment to be effective. The untreated wheat straw was having 14.1 % lignin which decreased to 9.0% on treatment with 1% sulfuric acid alongwith 1%Tween 20. The effect of increasing concentration of acid along with 1% Tween 20 on the different components of the wheat straw is presented in table 2. During the present investigation wheat straw was ground to size of 1mm and treated with 1 to 5% sulfuric acid alongwith 1% Tween 20 and found that pretreatment with 3% acid in addition to 1% Tween 20 led to 71.4% removal of lignin and 63.3% decrease in hemicellulose content. The cellulose content increased upto 55.5% after pretreatment. It was observed that the treatment with 3% acid along with 1% Tween 20 led to maximum removal of lignin maximum thereby facillating maximum recovery of cellulose. The hemicellulose content decreased with increase in acid concentration (3%). Therefore, for further studies the treatment of wheat straw with 3% dilute sulfuric acid and 1% Tween 20 was selected. Qi *et al.*, (2010) also found that the cellulose content in acid pretreated wheat straw varied between 53.1% and 58.5% and also concluded that the increase in the cellulose content was in agreement with the reduction in the hemicelluloses contents.

Table 1
Initial analysis of wheat straw

Substrate	*Cellulose % (w/w)*	*Hemicellulose % (w/w)*	*Lignin % (w/w)*
Untreated wheat straw (1 mm)	23.2	30.0	14.1

Table 2
Effect of sulfuric acid pretreatment on cellulose, hemicellulose and lignin content of wheat straw

Treatment (% Sulfuric acid + %Tween 20, w/v)	*Cellulose % (w/w)*	*Hemicellulose % (w/w)*	*Lignin % (w/w)*
1+ 1	26.5	24.0	9.0
2 + 1	32.0	21.0	6.0
3 + 1	55.5	11.0	4.0
4 + 1	55.4	10.9	4.0
5 + 1	55.0	9.9	3.8

Figure 1: Wheat straw

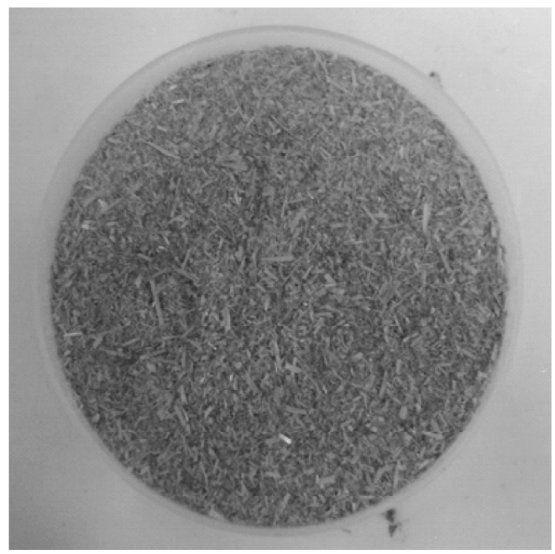

Figure 2: Wheat straw (1mm particle size)

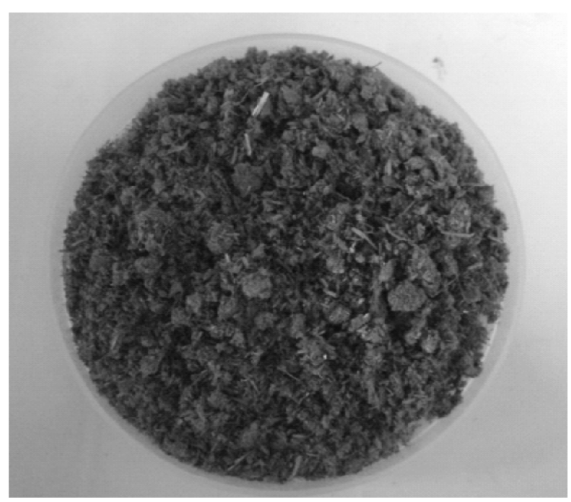

Figure 3: Wheat straw pretreated with 3% sulfuric acid along with 1% Tween 20

CONCLUSION

Wheat straw obtained from Directorate of Farm, CCS HAU, Hisar contained 23.2% cellulose, 30% hemicellulose and 14.1% lignin. After grinding to 1 mm size there was no change in composition of wheat straw. Pretreatment of powered wheat straw with 3% (w/v) sulfuric acid along with 1% (w/v) Tween 20 for 90 minutes in an autoclave at 121°C was found to be the best pretreatment in term of maximum hemicellulose solublization and lignin removal. The cellulose content increased upto 55.5% after pretreatment with 3% H_2SO_4 along with tween 20 treatment with the 71.4% removal of lignin and 63.3% decrease in hemicellulose content was observed. So it was concluded that wheat straw treated with 3% H_2SO_4 can be used for pretreatment of wheat straw because it increase cellulose content of wheat straw with the removal of lignin and makes the surface of wheat straw accessible for enzymatic action which is necessary step for bioethanol production. Acid pretreated wheat straw can be used for bioethanol after using hydrolysis and fermentation steps.

ACKNOWLEDGEMENTS

Thanks to Department of Microbiology, CCSHAU, Hisar for providing necessary facilities during research work.

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