International Journal of Research in Management, Science & Technology (E-ISSN: 2321-3264)
Vol. 5, No. 1, 81-85, April 17
Available at www.ijrmst.org

Impact of Particle Sizes of Wheat Straw on Hydrolytic Efficiency for Bioethanol Production

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Abstract- **Size reduction by mechanical processing (milling, grinding, extrusion, irradiation, etc.) is the vital step for bioethanol production by lignocellulosic biomass which enables the enzymes to attack substrate surface by decreasing crystallinity of cellulose and lignin content. The efficacy of enzymes for hydrolysis of cellulose can be improved by various pretreatments of the substrate. Results of this study showed that combination of physical (milling) and chemical (alkali-NaOH) pretreatment had high effect on the enzymatic hydrolysis of wheat straw. The optimal pretreatment condition was to grind the wheat straws to the sizes of 1-2 mm followed by treatment with 2.0% NaOH for 60 min in autoclave (121°C/15psi). The cellulose content of substrate was increased to 76.08% after pretreatment while the lignin content was decreased to 2.37%.**

Keywords- **pretreatment, crystallinity, enzymatic hydrolysis, cellulose, substrate.**

I. Introduction

Lignocellulosic biomass such as agriculture residue, wood and energy crops is an attractive material for bioethanol production, since it is the most abundant reproducible resource on earth which accounts for approximately 50% of biomass [1]. Cellulose is the principal polymer in composition of lignocellulosic biomass, with a small amount of hemicellulose and lignin [2], [3]. Wheat straw, rice straw, wheat bran and corn stover are the most favorable bioethanol producing feedstocks which could be an attractive alternative for disposal of these residues [4]. Wheat (*Triticum aestivum* L) straw, as an abundant by-product from wheat production, is generated at about 529 million tons worldwide every year [5], [6]. Effective bioconversion of lignocellulosic substrate into ethanol involves three major steps firstly, pretreatment– increase surface area and improves enzyme access to the cellulose; secondly, enzymatic hydrolysis– use of acid or enzymes (cellulases and hemicellulases); and thirdly, fermentation of released sugars by specialized organism to produce bioethanol [7]. Pretreatment is one of the most expensive processing steps within the conversion of biomass to fermentable sugar [8], [9]. In order to hydrolyze lignocellulosic biomass with enzymes successfully, the first step is to mechanically/physically process the feedstock (for example wood waste, straw, etc) to reduce size by shredding and grinding. Size of the substrate was kept upto 0.2-2mm by mechanical processing (viz. milling, grinding, extrusion, irradiation, etc) [10], [11], [12]. So, it is very important to use a suitable pretreatment method because crystallinity of cellulose, degree of polymerization (DP), moisture content, available surface area, and lignin content are factors that hinder the attack of enzymes [2], [13]. But this process is not economically feasible, as it consumes high energy to obtain preferred particle size [14], [15].

II. Materials and Methods

1. Chemicals

All chemicals used during this investigation were of AR and GR grade and were manufactured by Sigma, Himedia and E. Merck Ltd. etc.

2. Sample collection and Processing

Wheat straw was collected locally from crop fields of Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar. The substrate was first milled and sieved to size of >1mm, 1-2mm and 2-3mm. Then washed thoroughly with tap water to remove the dust particles and sun dried, carried to the lab and dried overnight in hot air oven at 70°C.It was stored in air tight containers at room temperature and further used for pretreatment.

3. Characterization of Wheat straw

Cellulose, hemicellulose, lignin content was estimated. Lignin and cellulose contents were estimated by determining the acid Detergent fiber (ADF) and Neutral detergent fiber (NDF) in the straw [16]. All experiments were performed in duplicate.

4. Pretreatment of wheat straw substrate

a) Physical pretreatment

Physical pretreatment included milling, grinding and chipping. It aimed to increase the surface area of biomass by reducing its particle size. This reduction facilitates the access of cellulases to the biomass surface increasing the conversion of cellulose.

b) Acid pretreatment

Concentrated Hydrochloric acid (HCl) was used to treat lignocellulosic material. The lignocellulosic biomass (wheat straw) at a solid loading of 10% (w/v) was pretreated in autoclave with hydrochloric acid with concentrations of 1, 2 and 3% (v/v) as wetting agent for residence time 60 minutes at 121°C and 15 psi. The collected residue was neutralized (pH-7) with deionized water and then dried at 70^{0}C for further analysis of cellulose, hemicellulose and lignin contents.

c) Alkali pretreatment

Alkali (Sodium hydroxide) was used for the pretreatment of lignocellulosic materials as an effective pretreatment agent. The lignocellulosic biomass wheat straw at a solid loading of 10% (w/v) pretreated in autoclave with sodium hydroxide at concentrations of 1 and 2% (w/v) as wetting agent for residence time 60 minutes at 121°C and 15 psi. After pretreatment, the residues were collected and washed extensively with deionized water until neutral pH. After neutralization, the slurry was filtered through a muslin cloth to separate residues and liquid.

III. Results and Discussions

Milling reduces the size of the materials and degree of crystallinity of lignocelluloses, which improves the susceptibility of these materials to enzymatic hydrolysis and enhances the efficiency of degradation. [17], [18] and [3].

1 Characterization of Wheat straw

Lignin, cellulose, hemicellulose, organic carbon, total nitrogen and ash content of untreated was estimated. Further, lignin, cellulose and hemicellulose content of HCl and NaOH pretreated wheat straw was also estimated.

Table 3.1 Lignin, cellulose and hemicellulose content in untreated wheat straw

Size	Lignin	Cellulose	Hemicellulose
>1mm	13.69	43.65	27.17
1-2mm	11.08	52.48	34.91
2-3mm	11.71	47.28	32.54

The lignin content of 13.69%, 11.08% and 11.71% was observed in >1mm, 1-2mm and 2-3mm sizes of untreated wheat straw respectively. Similarly, the cellulose and hemicellulose content of untreated wheat straw was observed to be 43.65%, 52.48%, 47.28% and 27.17%, 34.91%, 32.54% respectively (Table 3.1).

Lignin content was decreased from 13.69% to 11.08 with increase in size from >1mm to 1-2mm, but increases from 11.08% to 11.71% with further increase in size. Similar pattern is followed with Cellulose and hemicellulose content i.e. increased from 43.6% to 52.48% and 27.17% to 34.91% respectively with increase in size and decrease with further size increment. It is due to increased surface area and breakage of bonds between lignin and hemicellulose carbohydrate [19], [21].

2 Pretreatment of lignocellulosic biomass

a) Effect of HCl pretreatment on wheat straw

The effect of hydrochloric acid pretreatment on hemicellulose, lignin and cellulose removal was investigated by conducting experiments at different concentrations of hydrochloric acid on different sizes of wheat straw. The concentration of hydrochloric acid was 1%, 2% and 3% and the sizes of wheat straw was >1mm, 1-2mm and 2-3mm.

Table 3.2 Analysis of wheat straw substrate pretreated with 1% HCl

Size	Lignin	Cellulose	Hemicellulose
>1mm	4.2	57.78	18.01
1-2mm	2.4	65.97	27.27
2-3mm	4.4	53.37	22.7

When wheat straw pretreated with 1% HCl, there is increase in cellulose and hemicellulose 57.78% to 65.97% and 18.01% to 27.27% respectively at the sizes >1mm and 1-2mm, but decrease in lignin content from 4.2% to 2.4%. It was observed that when the size of substrate increases further i.e.1-2mm to 2-3mm, cellulose and hemicellulose decreases with increase in lignin content 65.97% to 53.37% and 27.27% to 22.7% and 2.4% to 4.4% respectively (Table 3.2). Pretreatment with dilute acid alters lignin-carbohydrate linkage whereas high acid concentration results in loss of carbohydrates [21].

Table 3.3 Analysis of wheat straw substrate pretreated of with 2% HCl

Size	Lignin	Cellulose	Hemicellulose
>1mm	8.4	65.9	18.01
1-2mm	5.18	69.3	23.85
2-3mm	8.2	66.75	20.61

When wheat straw pretreated with 2% HCl there is increase in cellulose and hemicellulose 65.9% to 69.3% and 18.01% to 23.05% respectively at the sizes >1mm and 1-2mm, but decrease in lignin content from 8.4% to 5.18%. The size of substrate increases further from 1-2mm to 2-3 mm, cellulose and hemicellulose decreases with increase in lignin content 69.3% to 66.75% and 23.85% to 20.61% and 5.18 % to 8.2% respectively (Table 3.3). Dilute acid pretreatment does not remove lignin from the substrate but only modifies lignin carbohydrate linkage [22]. Dilute acid solubilize hemicellulose and remain lignin and cellulose intact so that enzymatic digestibility of cellulose is enhanced [23].

Table 3.4 Analysis of wheat straw substrate pretreated with 3% HCl

Size	Lignin	Cellulose	Hemicellulose
>1mm	9.03	67.7	21.87
1-2mm	5.39	71.92	23.35
2-3mm	8.12	68.15	22.53

Wheat straw pretreated with 3% HCl, it is observed that there is increase in cellulose and hemicellulose 67.7% to 71.92% and 21.87% to 23.35% respectively at the sizes >1mm and 1-2mm, but decrease in lignin content from 9.03% to 5.39%. When the size of substrate increases further from 1-2 mm to2-3mm, cellulose and hemicellulose decreases with increase in lignin content 71.92% to 68.75% and 23.35% to 22.53% and 5.39% to 8.12% respectively (Table 3.4). Acid pretreatment removes the lignin sheath which disrupts the enzyme accessibility to cellulose making cellulose available for hydrolysis in free form [24], [25], [26].

b) Effect of NaOH pretreatment on wheat straw with various sizes

The effect of sodium hydroxide pretreatment on lignin, cellulose and hemicellulose removal was investigated by conducting experiments at different concentrations of sodium hydroxide on >1mm, 1-2mm and 2-3mm sizes of wheat straw. The concentration of sodium hydroxide was 1% and 2%.

Table 3.5 Comparative effect of NaOH pretreatment on lignin, cellulose and hemicellulose in different sizes of wheat straw

Wheat Straw Size (mm)	NaOH (%)					
	1%			2%		
	Lignin	Cellulose	Hemicellulose	Lignin	Cellulose	Hemicellulose
>1	4.23	69.6	23.27	3.84	73.15	23.35
1-2	3.67	71.34	25.03	2.37	76.08	21.27
2-3	4.78	68.13	24.36	3.78	71.26	24.77

When wheat straw was pretreated with 1% NaOH, the lignin content of 4.23%, 3.67% and 4.78% was observed in >1mm, 1-2mm and 2-3mm sizes respectively. It is clearly shown by the results that with much bigger size of substrate decreases its enzymatic hydrolysis efficiency, as the bounded particles of cellulose are not easily available to microbes. Similarly, the cellulose and hemicellulose content of 1% NaOH pretreated wheat straw was observed to be 69.6%, 71.34%, 68.13% and 23.27%, 25.03%, 24.36% respectively (Table 3.5). Alkaline pretreatment is basically a delignification process, in which a significant amount of hemicellulose is solubilized as well [23].

Secondly, it was observed that when wheat straw was pretreated with 2% NaOH, the lignin content of 3.84%, 2.37% and 3.78% was observed in >1mm, 1-2mm and 2-3mm sizes respectively. Similarly, the cellulose and hemicellulose content observed to be 73.15%, 76.08%, 71.26% and 23.35%, 21.27%, 24.77% respectively. The main effect of alkali pretreatment on lignocellulosic biomass is delignification by breaking the ester bonds cross linking lignin and xylan and glycosidic side chains degradation thus increasing the porosity of biomass and partial salvation of hemicellulose [27], [22], [29], [30] and [31] reported 51%, 65.63% and 67% increase in cellulose content in wheat straw, cotton stalk and sugarcane bagasse respectively after pretreatment with 2% sodium hydroxide. NaOH treatment causes swelling of substrate, leading to an increase in the internal surface area, a decrease in the degree of crystallinity and disruption of lignin structure [27]. The lignin decreased because of its solubilisation (break down of bonds) in the alkali aqueous solution [32]. Therefore, alkali pretreatment is contemplated as an efficient method for the removal of lignin from cellulosic biomass.

IV. CONCLUSION

Enzymatic hydrolysis is affected by various factors comprising the surface areas available, content of lignin present, crystallinity and degree of polymerization etc. The purpose of pretreatment is delignification and lessened hemicellulose, reduces cellulose crystallinity and increase the porosity. Therefore, selecting appropriate pretreatment methods plays a significant role in increasing the efficiency of cellulose hydrolysis. Pretreatment methods includes physical (milling, grinding, chopping) and chemical collectively gives very effective results (depends on kind of biomass).

ACKNOWLEDGMENTS

The authors would like to express their gratitude to University Grant Commission (UGC-BSR) for the financial support.

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