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Research Article

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Pretreatment of paddy straw for enhanced saccharification

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ABSTRACT

Present study deals with enhanced saccharification of paddy straw by optimizing various physical and chemical pretreatments such as steam explosion, temperature and treatment with hydrogen chloride, a highly electronegative acid. The amount of breakdown of lignin, hemi-cellulose & cellulose was determined using Fourier Transform Infrared Spectroscopy (FTIR). The maximum amount of breakdown of lignin is observed for 90minutes duration at wave number 1462.88cm^{-1} and maximum exposure of xylan is observed at wave number 1602.9cm^{-1}for 90 minutes exposure to steam explosion. In coupled pretreatment method (steam explosion & acidic simultaneously given) maximum cellulose breakdown took place after acidic pretreatment followed by steam explosion which was observed by FTIR at 1320.32cm^{-1} wave number simultaneously histological changes were determined by staining and visualization at 10X using saffranin dye. In the qualitative analysis using High Pressure Liquid Chromatography of sample after 90 minutes steam explosion chromatogram of Xylitol (Rt = 9.886) & 60 minutes acidic pretreatment chromatogram of, Xylose (Rt = 8.741) were observed.

Keywords: lignocellulosic, optimization, saccharification, steam explosion

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INTRODUCTION

Biomass with high lignocellulosic content has the maximum potential for the production of biofuels like: Butanol, Ethanol etc. Conversion of lignocelluloses' to a biofuel is multistep process which includes the following processes: Mechanical Disintegration, Physical pretreatment, Chemical Hydrolysis, Enzymatic saccharification & Fermentation. In comparison to all of the steps pretreatment is the most important steps against the recalcitrant characteristic of lignin which shield the hemi-cellulose and cellulose from the microbial activity [1, 2].

The fact that pretreatment is given before fermenting sample it is to carry out saccharification of disaccharides and carbohydrates present in lignocelluloses biomass. Pretreatment methods refer to the solubilization and separation of one or more of these components of biomass. It makes the remaining solid biomass more accessible to further chemical or biological treatment [3].

Lignin is the most recalcitrant towards degradation in comparison to cellulose and due to its crystal structure it proves to be resistant to hydrolysis. Although weak acids can remove lignin but leads to poor hydrolysis of cellulose whereas concentrated acids causes corrosion under high temperature conditions which is very expensive technique [4, 5].

Physical pretreatment decreases the crystallinity of cellulose and its particle size. Also, promotes softening and partial de-polymerization of lignin. Partially hydrolysis of hemi-cellulose is also supported by it. These methods are reported to be cost effective and useful at large scale. [6] Concentrated acids like: HCl & H_2SO_4 hydrolyze the hemi-cellulosic & cellulosic content of lignocellulosic biomass whereas lignin is left unaffected. Its main advantage is the high yield of monomeric sugar, but major drawback of using concentrated acid is their corrosive nature. [7] Amount of total reducing sugar released in hydrolysate helps in the determination of effect of type of pretreatment on rice husk. It’s been observed that acid pretreatment yielded higher Total Reducing Sugar than alkali pretreatment with HCl at the highest yield of 15.04mg/mL followed by HNO_3 and H_2SO_4 respectively. [8, 9] Acid pretreatment is considered as one of the most important techniques and aims for high yields of sugars from lignocelluloses. It is usually carried out by concentrated or diluted Sulfuric acids (usually in the ratio of 1:6 w/v) at temperatures between 130 °C for approx. 3hrs. Then the extracted pulp is washed repeatedly with distilled water to adjust its pH to 4.8 [10, 3]. Physical & Chemical pretreatment of lingo-cellulose is usually a short process step which involves only a few minutes at high temperature. Controlling and putting a check on the breakdown of lignin requires rapid and accurate methods of analysis by common methods like wet chemical analysis and HPLC however these are time consuming and may take several days [11]. Fourier transform infrared (FTIR) spectroscopy is rapid and quantitative technique for the identification of components of solid or lingo-cellulose in the mid-IR region [12].

Using carbohydrate columns of HPLC, with the careful manipulation of mobile phase baseline resolution of known monomeric sugars and dimeric sugars like xylose, fructose and manose etc. were analyzed in significant quantities in hydrolyaste of biomass. [13]

EXPERIMENTAL SECTION

2.1 Raw Material: Rice straw was obtained from local producer in Phagwara, Punjab, India. Straw was washed, air dried & then grounded using mixer grinder (particle size: 0.1-0.5mm). Then sieved by pore size of 0.5mm and stored at room temperature.

Raw material was divided into 4stacks of 20gm each.

2.2 Physical Pretreatment: Straw was soaked in 400mL of distilled water in 2L beaker in a ratio of 1gm of straw per 20mL of water. This step was performed for 3 stacks of 20gm and each soaked in distilled water. Then steam explosion (wet oxidation) was done by autoclaving each of the three stacks at 121°C (15psi) but at varied time durations of 40, 60 & 90 minutes respectively. Hydrolysate water was filtered and stored at cool temperature and then filtrate was air dried and stored.

2.3Quantitative Analysis: Amount of lignin degradation of pretreated raw material was quantified by using FTIR spectroscopy (Fourier Transform Infrared Spectroscopy). Exposure to cellulose and hemi-cellulose helps in determining the degradation of lignin. The quantification of amount of cellulose and xylan degradation provides an approach for the optimization of effective duration of physical pretreatment. This step was repeated after chemical pretreatment for the first and the fourth stack in order to quantify the effect of acid exposure in lignocellulosic content degradation using FTIR.

Ranges for Lignin, Xylan & Cellulose are as follows:

S.No.	Content	Wave number Range	Band Assignment	Reference
1.	Lignin	1508-1420	Aromatic skeletal vibrations	[14]
2.	Xylan	1600-1670	C=O stretch	[15]
3.	Cellulose	1316-1384	CH_2 wagging vibration	[16]

2.4 Chemical Pretreatment: Acidic hydrolysis of raw material was carried out by treating raw material with 1N hydrochloric acid in the ratio of 1:8 gm/mL this step was followed for first stack which was steam exploded and then for the fourth stack with no pre-exposure to steam. The pH of hydrolysate was balanced to neutral and stored in cool temperature.

2.5 Qualitative Analysis: Qualitative analysis for observing the effect of each pretreatment step at structural level was carried out by histological determination using saffranin stain and observing the components under light microscope at 10X magnification. The quantitative analysis for observing the wastage of monomers of sugars in hydrolysate was carried out by High Pressure Liquid Chromatography (HPLC) using carbohydrate columns.

RESULTS AND DISCUSSION

The main focus of present study was to optimize the time duration for steam explosion (wet oxidation) as a physical pretreatment method for maximum degradation of lignocellulosic content with least wastage of monomers of sugar during hydrolysate removal.

3.1 Quantified comparison of lignocellulosic degradation after each pretreatment step: Comparison of each step for their efficiency in lignin removal and cellulose exposure is determined by Fourier Transform spectroscopy (FTIR).

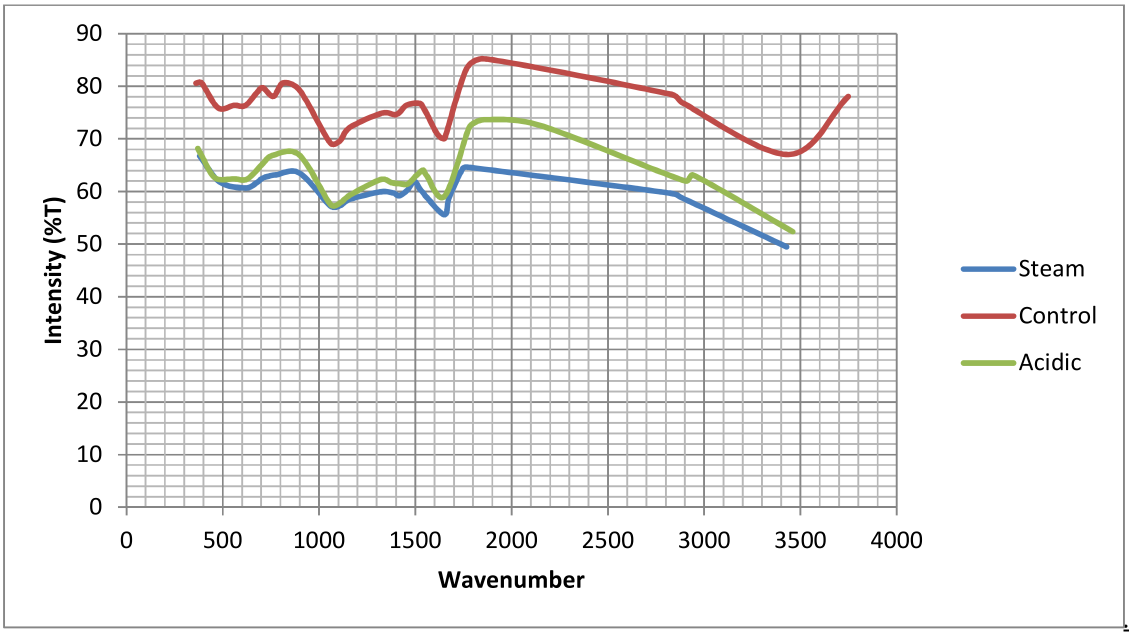

Fig.1: Stepwise coupled pretreatments comparative analysis of sample using FTIR analysis for determination of Ligno-cellulose breakdown

In coupled pretreatment method (steam explosion and acid treated was given simultaneously), maximum cellulose breakdown took place after acidic pretreatment followed by steam explosion which was observed by FTIR at 1320.32cm^{-1} wave number. Simultaneous treatment of biomass with steam explosion and acid gives more beneficial result in comparison to individual treatment (Fig 1).

3.2 Optimization of duration for steam explosion: Optimization of duration of steam explosion is done by the comparative analysis of FTIR results at duration of 40, 60 & 90 minutes respectively (Fig 2).

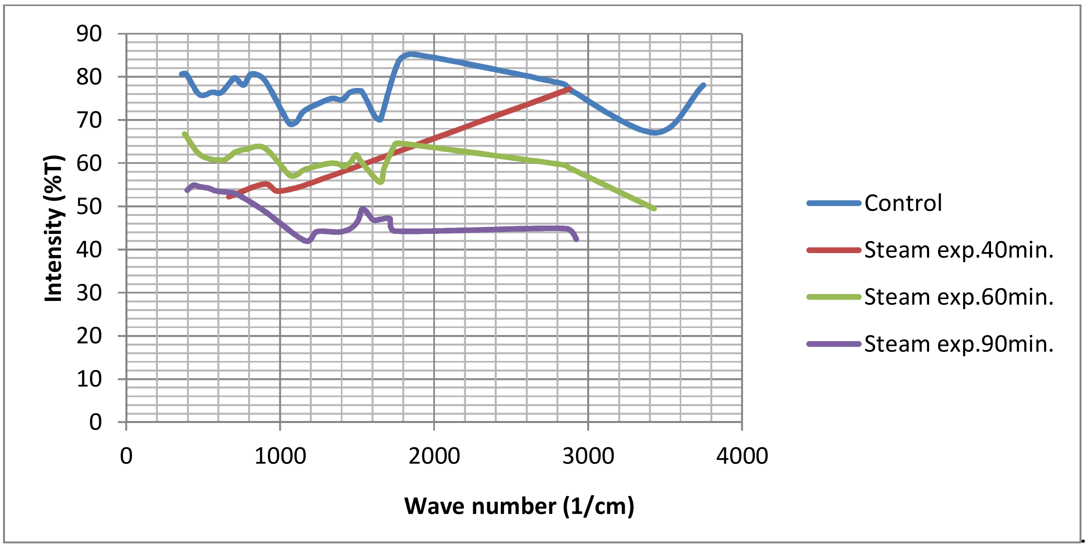

Fig.2: Comparative analysis of sample using FTIR analysis for determination of ligno-cellulose breakdown by steam explosion at different duration

Maximum amount of breakdown of lignin is observed after 90 minutes of duration at wave number 1462.88cm^{-1} and maximum exposure of xylan is observed at wave number 1602.9cm^{-1}for 90 minutes and minimum breakdown is observed in case of 40minutes exposure to steam explosion. Exposure duration for 90 minutes leads to the maximum degradation of lignin as a result exposing most of the hemi-cellulosic and cellulosic content of biomass which can be easily degraded with maximum yield of glucose monomers.

3.3 HPLC chromatogram for hydrolysate content analysis: Determination for the presence (wastage) of monomers of sugar in hydrolysate after each pretreatment.

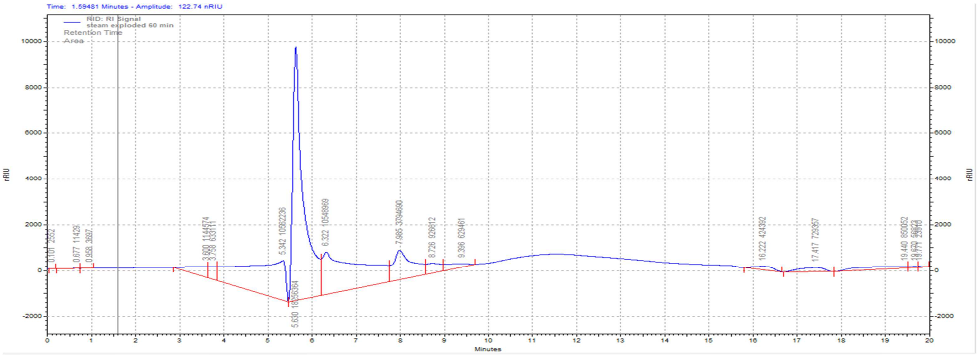

Fig3: Analysis of hydrolysate from steam exploded (60 minutes) sample by HPLC

No known chromatogram was observed at 60 minutes in the hydrolysate of steam exploded sample which could be inferred to steam explosion for the duration of 60 minutes, has no noticeable effect on the structure of lignocellulosic biomass.

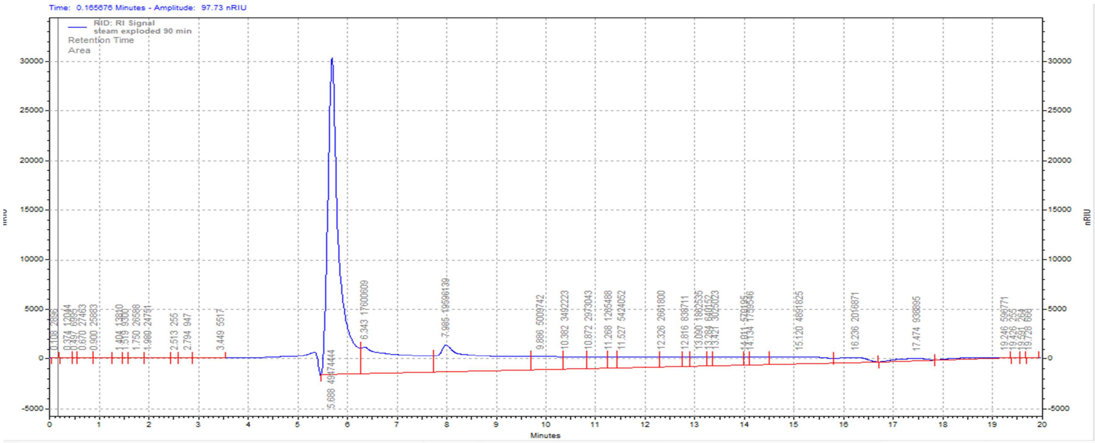

Fig4: Analysis of hydrolysate from steam exploded (90 minutes) sample by HPLC

Chromatogram of xylitol (Rt = 9.886) and glycerol (Rt = 11.527) was observed at 90 minutes steam exploded sample's hydrolysate which could be attributed that breakdown of lignin taking place as glycerol one of the main contents of lignin and presence of xylitol indicates the exposure of hemi-cellulose(Fig. 4).

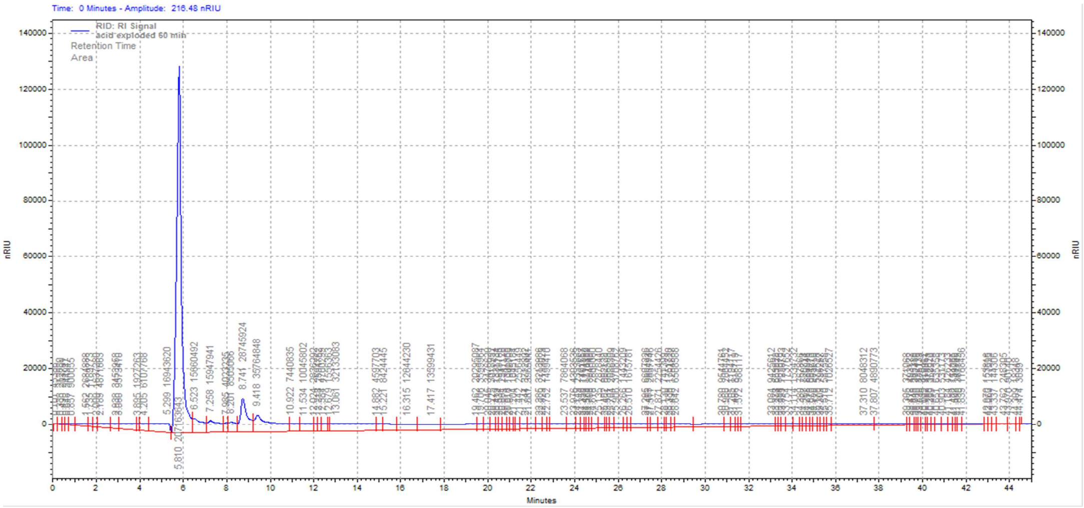

Fig5: Analysis of hydrolysate from acid exploded (60 minutes) sample by HPLC

Chromatogram of xylose (Rt = 8.741) was observed at 60 minutes acid exploded (1N HCl) sample's hydrolysate. Hydrolysis of sample with 1N HCl resulted less wastage of monomers of glucose with no inhibitor such as furfural that was observed in comparison to other types of acidic pretreatment. Chromatogram of xylose evidenced positively the result of FTIR which showed the exposure and breakdown of lignin and hemi-cellulose (Fig.6).

3.4 Histological determination of Ligno-cellulosic content of rice straw:

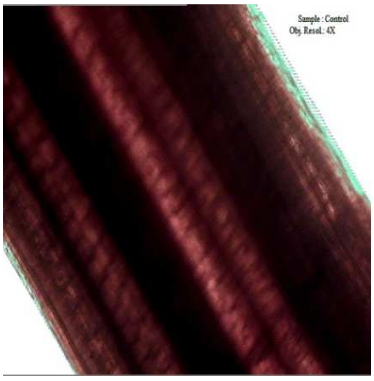

Fig.6 (a) Control

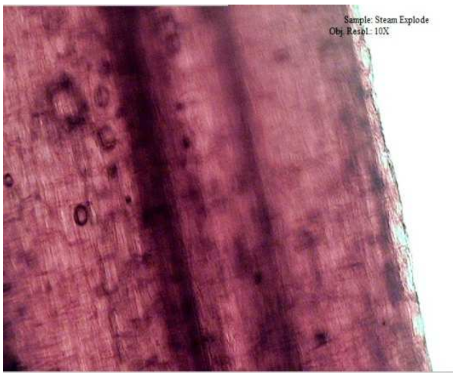

Fig6 (b) Steam Exploded

Fig.6 (a) Histological determination of control sample under microscope at 10X using saffranin stain **(b)** Histological determination of Steam exploded sample under microscope at 10X using saffranin stain

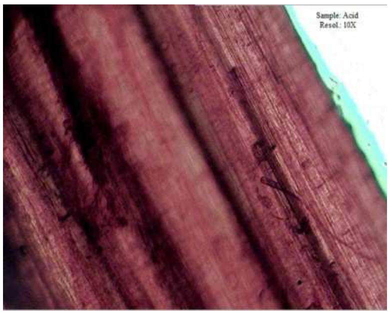

Fig.6 (c) Acid Hydrolyzed

Fig.6 (c) Histological determination Acid Hydrolyzed (HCl 1N) sample using saffranin dye under microscope at (10X)

Light Yellowish Pink observed: Cellulose & Hemi-cellulose

Dark Red bands observed: Lignin

On comparing histological figures (6 a), (6 b) and (6 c) of control, steam exploded and acid hydrolyzed sample, it is inferred that maximum breakdown of lignin and hemicellulose took place during acid hydrolysis then in steam exploded sample. The histological observation provides a visual approach to the stepwise changes taking place in the structural composition of biomass.

CONCLUSION

Lignocellulosic degradation for the formation of monomers of glucose has always been an initial step for bio-fuel production processes. Whereas effective degradation of lignocellulosic biomass can only be achieved by cheap and effective pretreatment or saccharification processes. After optimizing the steam explosion process for enhanced pretreatment it can be concluded that maximum breakdown of Lignin was occurred at the duration of 90 minutes. Degradation of hemicelluloses into Xylose is observed after acidic explosion pretreatment for 60 minutes with no furfural inhibitor formation. It can be concluded from the following study that to obtain the maximum degradation lignin in future raw material must be steam exploded for 90 minutes at 121°C|15psi and then followed by simultaneous acidic explosion with 1N hydrochloric acid for 60 minutes at 121°C|15 psi.

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