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Study on Filtrate Obtained from Batch-Wise Pretreatment of Wheat Straw at Different Concentrations of H_2SO_4 and Autoclave Time

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Abstract: The objective of this study was to investigate the optimum conditions for maximum delignification of wheat straw for production of fuel grade bioethanol. Pretreatment of wheat straw was conducted in eight batches at 121°C, 15 lb and autoclave time 15, 30, 45, 60, 75, 90, 105 and 120 min separately. Each batch consisted of six 250 ml conical flasks (F_1, F_2, F_3, F_4, F_5 and F_6) containing 10 g of wheat straw. In flask F_1 100 ml distilled water (0.0% H_2SO_4) was added and in flasks F_2-F_6 100 ml of H_2SO_4 solution of Conc. 0.2, 0.5, 1.0, 1.5 and 2.0% was added respectively. Filtrate obtained from each batch was used for the estimation of Total sugars, Reducing sugars and Total Phenols accordingly. The results showed that pretreatment of wheat straw with dil. H_2SO_4 in Flask (F_5) of batch-V was promising at 1.5% H_2SO_4 for 75 min and total phenols in filtrate were 10.89 mg/ml and weight loss was 49.99%. Pretreatment of wheat straw with pure water (0.0% H_2SO_4) at 121°C, 15 lb for 45 min (Batch-III, flask F_1) showed significant delignification and under these conditions maximum production (mg/ml) of total sugars (36.414), Reducing sugar (33.015) and Total phenol (7.58) were found. Comparitively delignification was 43.66% more with 1.5% H_2SO_4 for 75 min than pure water (0.0 % H_2SO_4) for 45 min.

Key words: Pretreatment · Lignocellulose · Wheat straw · Filtrate analysis · Bioethanol · Biofuel

INTRODUCTION

The present increased concern for the security of the oil supply and the negative impact of fossil fuels on the environment, particularly greenhouse gas emissions, has put pressure on society to find renewable fuel alternatives. Most common renewable fuel, bioethanol is produced from lignocelluloses of plant biomass [1] Second generation bioethanol production uses relatively cheap, abundant and renewable agricultural by-products, such as wheat straw, rice straw, grasses or forestry residues. Wheat straw is nowadays is the potential lignocellulosic raw material for fuel-ethanol production as an alternative to starch or sugar-containing feedstock. Wheat straw is a likely candidate for use in second generation bioethanol production [2]. Baig *et al.* [3] reported that lignocellulosic biomasses require removal of lignin by pretreatment to improve cellulose accessibility to cellulolytic enzymes for maximum saccharification. Usually this entails a heat treatment in water in presence of a catalyst (acid or base). A common pretreatment uses dilute sulfuric acid at 100-200°C to disrupt the lignin-carbohydrate matrix and to facilitate enzymatic cellulose hydrolysis [4-5].

During hot acid pretreatment, some of the polysaccharides are hydrolyzed, mostly hemicellulose. The resulting free sugars can degrade to furfural (from pentoses) and to 5-hydroxymethylfurfural (HMF) from hexoses [6-7]. These compounds inhibit yeast cells and lead to decreased growth rate, ethanol production rate and ethanol yield. In addition, their production means loss of fermentable sugars [8]. The goal of the pretreatment process is to break down the lignin structure and disrupt the crystalline structure of cellulose, so that the acids or enzymes can easily access and hydrolyze the cellulose [9]. Pretreatment can be the most expensive process in biomass-to-fuels conversion but it has great potential for improvements in efficiency and lowering of costs through

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further research and development [10]. Pretreatment must meet the following requirements: (i) improve the % age of cellulose (ii) decrease the % age of lignin (iii) less formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes and (iv) be cost-effective. Pretreatment methods are of different categories: physical, physicochemical, chemical (alkali, dilute acid, etc), biological, electrical, or a combination of these [11].

Dil. Sulfuric acid method of pretreatment of wheat straw is effective and promising at commercial level because ethanol production is more. H_2SO_4 is used for pretreatment of wheat straw to improve downstream enzymatic hydrolysis. Based on the dose of acid used in the process, it could be identified as concentrated and/or dilute-acid hydrolysis. Concentrated acid treatment has drawbacks including high acid and energy consumption, equipment corrosion and longer reaction time as well as obligation for acid recovery after treatment that largely limit its application [12-13]. In the second approach, low-concentration acids e.g. 0.5-1% H_2SO_4 and high temperatures are exploited. High temperature is favorable to attain acceptable rates of cellulose conversion to glucose. Despite low acid concentration and short reaction time, application of high temperatures in dilute-acid hydrolysis accelerates the rate of hemicellulose sugar decomposition and increases equipment corrosion [12, 14]. The main drawbacks of this method are formation of many inhibiting by-products and pH neutralization requirement for downstream processes [13,].

In our work, the optimization of process variables (Concentration and residence time) for pretreatment of wheat straw were addressed by means of design of eight batch wise experiments [16]. Pretreatment is an important and compulsory tool for biomass-to-biofuel conversion processes and is the subject of this paper. As Pakistan is an agricultural state and wheat straw is easily available at low price. Development of the process will strengthen the technological base of country in addition to saving foreign exchange spent on import of fossil fuel. This project will also produce technical man power which will be helpful for the development of biofuel industry in Pakistan which is the need of the day.

MATERIALS AND METHODS

Lignocellulosic Biomass: Wheat straw of the wheat (*Triticum aestivum* L.) Crop-2010 grown at Mareywala khoh village SOHIAN of District Gujranwala-Pakistan was used as a source of Lignocellulosic biomass. Wheat straw was washed to remove the undesired particles and dried. After drying the powder form (1-2mm) with hammer beater mill was prepared.

Pretreatment Method: Pretreatment of wheat straw was done in eight batches(I, II, III---VIII) at 121°C, 15 lb for varying time intervals (15, 30, 45, 60, 75, 90, 105 and 120 min). In each batch 10gm of wheat straw was taken in each of six conical flasks (F_1, F_2, F_3, F_4, F_5 and F_6). In flask F_1 100 ml distilled water (0.0%H_2SO_4) was added and in flasks F_2-F_6 100 ml of H_2SO_4 solution of Conc. 0.2, 0.5, 1.0, 1.5 and 2.0% respectively was added. Flasks were plugged with cotton and covered with Aluminum file. Six flasks of each batch were autoclaved at 121C, 15 lb for respective time i.e. 15, 30, 45, 60, 75, 90, 105 and 120 min. After autoclaving slurry was filtered. The filtrate was used for analysis (total sugars, reducing sugars and total Phenols). The residue was packed in muslin cloth bags and washed for 4 to 5 times using distilled water till neutral pH and oven dried. These oven dried samples were packed in plastic zip-bags and were stored for the estimation of lignin and cellulose content after Pretreatment [17].

Total Sugars Estimation: Total sugars in the filterate were estimated using the Method of Dubois *et al.* (1956) [18].

Reducing Sugars Estimation: Reducing sugars in the filterate were estimated using 3, 5-Dinitrosalysilic Acid (DNS) Method (1959) [19].

Total Phenols Estimation: Total phenolic compounds released during pretreatment were estimated as described by Carralero *et al.* [20] with slight modifications. In 20 ml test tube 0.25 ml filterate 1.25 ml Folin Reagent (diluted to 1:10 ratio) was taken and 1.0 ml of 7.5 % Na2CO3 was added. The reaction mixture was kept at room temperature for 2hr and then optical density (OD) was measured at 765 nm (Spectro UV-VIS, UVS-2800 Lambomed, USA). Vanillin (5mM) was taken as standard.

Cellulose Estimation: Cellulose in the untreated wheat straw was estimated by taking 1.0 g wheat straw sample (W_1) into round bottom flask. 15 ml of 80% acetic acid and 1.5 ml of concentrated HNO_3 was added in the sample and heated on electric reflux for 30 min. Then diluted, filtered and washed the residue by distilled water. The residue was taken in the crucible and dried it in the oven at 105°C over night. Weighed (W_2) the dried sample. Added Conc. HNO_3 enough to wet the dried residue, did charring and placed it in the Muffle furnace at 550°C for 4 hours. Then weighed the Ash (W_3) [21]. Cellulose % age was calculated using the following formula.

$$\% \text{ Age of Cellulose} = W_2\text{-}W_3/W_1 \times 100$$

Lignin Estimation: The percentage of lignin content in untreated samples was measured by considering lignin as remaining solid residue after hydrolysis with 1.25% H_2SO_4 for two hour and 72% H_2SO_4 hydrolysis for four hours. The slurry was filtered and residue was washed with distilled water to remove H_2SO_4 and oven dried at 105°C for constant weight. The lignin was expressed by using the formula [22]

$$\text{Lignin (\%)} = \frac{\text{Lignin Weight (g)}}{\text{Bagass Weight (g)}} \times 100$$

Protein Estimation: Total crude protein in untreated wheat straw was estimated by Kjeldahl method (N × 6.25). The method was developed in 1883 by Johan Kjeldahl.

Ash Estimation: Ash of the wheat straw was calculated according to the standard method (AOAC, 2005)[23].

Moisture Estimation: Moisture content of wheat straw was determined by gravimetric method [24].

RESULT AND DISCUSSION

Proximate Analysis of Untreated Wheat Straw: Proximate analysis of untreated wheat straw was conducted and presented in Fig 1 and results showed that Pakistani variety of wheat straw contains (% w/w) Cellulose 36.0, Hemicellulose 27.9, Lignin 22.0, Moisture 7.8, Protein 3.1 and Ash 3.2% (w/w). According to Prasad *et al.* [25] Cellulose, Hemicellulose and Lignin content of wheat straw are in the range of 33-40, 20-25 and 15-20 (%w/w), respectively. Our result are comparable to Saha *et al.* [15] who reported 27.7% Hemicellulose and 3.48% crude protein (w/w) in the wheat straw.

Fig 1 Proximate analysis of untreated wheat straw

Component	Content (% w/w)
Cellulose	36.0
Hemicellulose	27.9
Lignin	22.0
Moisture	7.8
Protein	3.1
Ash	3.2

Percentage of Hemicellulose = 100-Sum of all other components.

Batch Wise Analysis Of Filtrate Obtained After Pretreatment of 10 g Of Wheat Straw At 121°C, 15 lb for Different Time Period (15, 30, 45-120 min)

Batch-I: Analysis of Filtrate Obtained after Pretreatment of Wheat Straw at 121°C, 15 lb for 15 min: Total sugars estimated were 29.60 mg/ml when pure water (0.0% H_2SO_4) was used but when dil acid was used then total sugars were minimum 30.64 mg/ml at 0.2% H_2SO_4 and maximum 37.89 mg/ml at 1.5% H_2SO_4. Reducing sugars were 23.70 mg/ml when pure water (0.0% H_2SO_4) was used. But Reducing sugars were 24.82 mg/ml at 2.0% H2SO4 and maximum 46.80 mg/ml at 0.5% H_2SO_4. Total phenols estimated in filterate obtained after pretreatment of wheat straw at 121°C, 15 lb for 15 minutes were 5.06 mg/ml when pure water (0.0% H_2SO_4) was used. 07.47 mg/ml of 0.2% $H_2SO_4$and maximum 10.20 mg/ml at 1.0% H_2 SO_4.

Batch-II: Analysis of Filterate Obtained after Pretreatment of Wheat Straw at 121°C, 15 lb for 30 min: Total sugars estimated were 34.43 mg/ml when pure water (0.0% H_2SO_4) was used. When dil.acid was used then total sugars were minimum 21.73 mg/ml at 0.2% H_2SO_4 and maximum 44.68 mg/ml at 1.0% H_2SO_4. Reducing sugars were 26.731 mg/ml when pure water (0.0% H_2SO_4) was used. But reducing sugars were 35.74 mg/ml at 0.2 % H_2SO_4 and maximum 40.31 mg/ml at 1.0 % H_2SO_4. Total phenol sugars estimated in filterate obtained after pretreatment of wheat straw at 121°C, 15 lb for 30 minutes were 4.73 mg/ml when pure water (0.0% H_2SO_4) was used.7.47 mg/ml at 0.2% H_2SO_4 and maximum10.87 mg/ml at 1.0% H_2SO_4. Average of reducing sugars estimated in this batch (36.774 ml/gm) was highest of all the eight batches.

Batch-III: Analysis of Filtrate Obtained after Pretreatment of Wheat Straw at 121°C, 15 lb for 45 min: Table 3 Presents That total sugars estimated were 36.414 mg/ml when pure water (0.0% H_2SO_4) was used. When dil. acid was used then total sugars were minimum 29.71 mg/ml at 0.2% H_2SO_4 and maximum 58.00 mg/ml at 1.0% H_2SO_4. Reducing sugars were 33.015 mg/ml when pure water (0.0% H_2SO_4) was used. But Reducing sugars were 24.162 mg/ml at 1.5 % H_2SO_4 and maximum 40.15 mg/ml at 1.0% H_2SO_4. Total phenolic sugars estimated in filterate obtained after pretreatment of wheat straw at 121°C, 15 lb for 45 minutes were 7.58 mg/ml when pure water (0.0% H_2SO_4) was used, 07.30 mg/ml with 0.2 % H_2SO_4 and maximum 10.74 mg/ml with 1.0% H_2SO_4. Average total phenols of all six experiments were 7.85 mg/ml.

Table 1: Batch-I; Analysis of filtrate obtained after pretreatment of 10g of wheat straw at 121°C, 15 lb for 15 min

Sr. NO.	Conc. of H_2SO_4 (%)	Dry Wt. of Residue (g)	Total sugars (mg/ml)	Reducing sugars (mg/ml)	Total phenols (mg/ml)
F_1	0.0	7.054	29.60	23.70	5.06
F_2	0.2	6.666	30.64	39.83	7.47
F_3	0.5	6.113	41.39	46.80	8.67
F_4	1.0	5.370	34.85	41.50	10.20
F_5	1.5	5.769	37.89	31.85	9.80
F_6	2.0	5.350	35.74	24.82	8.74

Table 2: Batch-II; Analysis of filtrate obtained after pretreatment of 10g of wheat straw at 121°C, 15 lb for 30 min

Sr. NO.	Conc. of H_2SO_4(%)	Dry Wt. of Residue (g)	Total sugars (mg/ml)	Reducing sugars (mg/ml)	Total phenols (mg/ml)
F_1	0.0	6.779	34.43	26.731	4.73
F_2	0.2	7.400	21.73	35.74	7.38
F_3	0.5	6.350	47.57	38.32	9.40
F_4	1.0	6.003	44.68	40.31	10.54
F_5	1.5	5.570	44.65	39.20	10.87
F_6	2.0	5.570	43.54	40.30	10.54

Table 3: Batch-III; Analysis of filtrate obtained after pretreatment of 10g of wheat straw at 121°C, 15 lb for 45 min

Sr. NO.	Conc. of H_2SO_4 (%)	Dry Wt. of Residue (g)	Total sugars (mg/ml)	Reducing sugars (mg/ml)	Total phenols (mg/ml)
F_1	0.0	6.399	36.414	33.015	7.58
F_2	0.2	6.982	29.71	38.13	7.30
F_3	0.5	6.143	54.62	39.23	9.37
F_4	1.0	5.546	58.00	40.15	10.74
F_5	1.5	5.245	50.49	39.70	10.01
F_6	2.0	5.082	49.25	24.162	9.01

Table 4: Batch-IV: Analysis of filtrate obtained after Pretreatment of 10g of wheat straw at 121°C, 15 lb for 60 minutes

Sr. NO.	Conc. Of H_2SO_4 (%)	Dry Wt. of Residue (g)	Total sugars (mg/ml)	Reducing sugars (mg/ml)	Total phenols (mg/ml)
F_1	0.0	6.894	35.78	27.34	4.03
F_2	0.2	6.773	54.73	37.08	7.38
F_3	0.5	5.954	24.86	39.34	9.40
F_4	1.0	5.374	61.21	38.13	10.48
F_5	1.5	5.098	50.05	38.73	10.87
F_6	2.0	5.073	38.12	17.71	10.42

Batch-IV: Analysis of Filtrate Obtained after Pretreatment of Wheat Straw at 121°C, 15 lb for 60 min: Total sugars estimated were 35.78 mg/ml when pure water (0.0% H_2SO_4) was used. When dil. H_2SO_4 acid was used then total sugars were minimum 24.86 mg/ml at 0.5% H_2SO_4 and maximum 61.21 mg/ml at 1.0% H_2SO_4. Reducing sugars were 27.34 mg/ml when pure water (0.0% H_2SO_4) was used. But Reducing sugars were 17.71 mg/ml at 2.0% H_2SO_4 and maximum 39.34 mg/ml at 0.5% H_2SO_4. Total phenolic sugars estimated in filterate obtained after pretreatment of wheat straw at 121°C, 15 lb for 60 minutes were 4.03 mg/ml when pure water (0.0% H_2SO_4) was used, 07.38 mg/ml at 0.2 % H_2SO_4 and maximum10.87 mg/ml when 1.5% H_2SO_4 was used.

Batch-V: Analysis of Filtrate Obtained after Pretreatment of Wheat Straw at 121°C, 15 lb for 75 min: Total sugars estimated were 6.30 mg/ml when pure water (0.0% H_2SO_4) was used. When dil. acid was used then total sugars were minimum 35.67 mg/ml at 1.0% H_2SO_4 and maximum 65.34 mg/ml at 1.5% H_2SO_4. Average of total sugars of the six experiments at 0.0, 0.2, 0.5, 1.0, 1.5, 2.0 % H_2SO_4 was 39.64 mg/ml (Table 5). Reducing sugars were 0.71 mg/ml when pure water (0.0% H_2SO_4) was used. But reducing sugars were 10.98 mg/ml at 0.2% H_2SO_4 and maximum 42.99 mg/ml at 1.5% H_2SO_4. Average of Reducing sugars Of the six experiments at 0.0, 0.2, 0.5, 1.0, 1.5, 2.0% H_2SO_4 was 29.68 mg/ml. Total phenolic sugars estimated in filtrate obtained after pretreatment of wheat

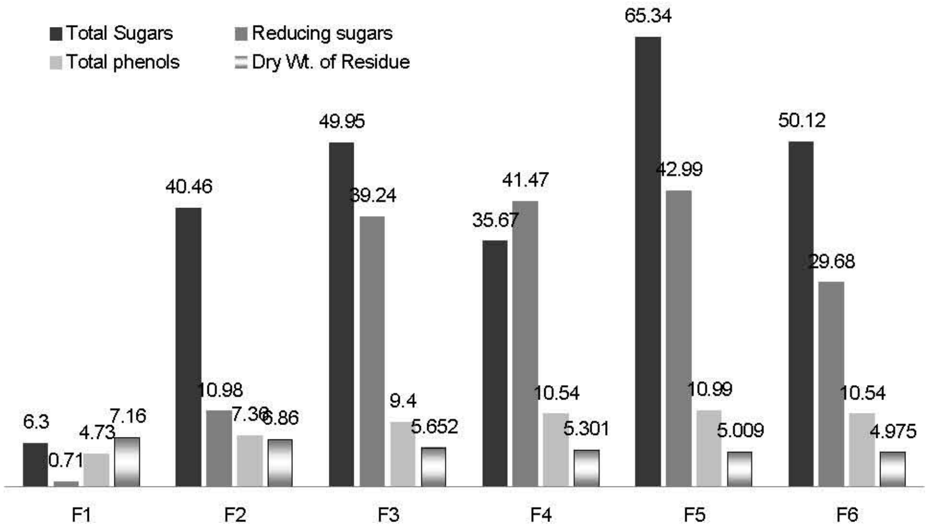

Fig. 2: Batch-V; Analysisof filtrate obtained after pretreatment of wheat straw at 121°C, 15 lb for 75 min

Table 5: Batch-V; Analysis of filtrate obtained after pretreatment of 10g of wheat straw at 121°C, 15 lb for 75 min

Sr. NO.	Conc. Of H_2SO_4 (%)	Dry Wt. of Residue (g)	Total sugars (mg/ml)	Reducing sugars (mg/ml)	Total phenols (mg/ml)
F_1	0.0	7.160	6.30	0.71	4.73
F_2	0.2	6.862	40.46	10.98	7.36
F_3	0.5	5.652	49.95	39.24	9.40
F_4	1.0	5.301	35.67	41.47	10.54
F_5	1.5	5.009	65.34	42.99	10.89
F_6	2.0	4.975	50.12	42.70	10.54

straw at 121°C, 15 lb for 75 minutes were 4.73 mg/ml when pure water (0.0% H_2SO_4) was used, 07.36 mg/ml at 0.2% H_2SO_4 and maximum10.89 mg/ml when 1.5% H_2SO_4 was used (Fig. 2). Average total phenols of all six experiments were 8.93 mg/ml. weight loss of residue was highest of all the eight batches in the flask F_5 due to maximum delignification and highest amount of total phenols was estimated in F_5 of batch-V.

Batch-VI: Analysis of Filtrate Obtained after Pretreatment of Wheat Straw at 121°C, 15 lb for 90 min: Total sugars estimated were 3.72mg/ml when pure water (0.0% H_2SO_4) was used. When dil. acid was used then total sugars were minimum 42.88 mg/ml at 0.2% H_2SO_4 and maximum 57.98 mg/ml at 2.0% H_2SO_4. Reducing sugars were 0.41 mg/ml when pure water (0.0% H_2SO_4) was used. But Reducing sugars were 23.37 mg/ml at 0.2% H_2SO_4 and maximum 42.90 mg/ml at 1.5% H_2SO_4. Total phenolic sugars estimated in filterate obtained after pretreatment of wheat straw at 121°C, 15 lb for 90 minutes were 7.28 mg/ml when pure water (0.0% H_2SO_4) was used. Total Phenols were 9.37 mg/ml at 0.2% H_2SO_4 and maximum10.74 mg/ml at 1.0% H_2SO_4.

Batch-VII: Analysis of Filtrate Obtained after Pretreatment of Wheat Straw at 121°C, 15 lb for 105 min: Results in Table-7 showed that total sugars estimated were 0.59 mg/ml when pure water (0.0% H_2SO_4) was used. But when dil. acid was used then total sugars were minimum 16.73 mg/ml at 0.2% H_2SO_4 and maximum 48.11 mg/ml at 1.5% H_2SO_4. Reducing sugars were 03.05 mg/ml when pure water (0.0% H_2SO_4) was used. But Reducing sugars were 04.90 mg/ml at 0.5% H_2SO_4 and maximum 42.39 mg/ml at 1.5% H_2SO_4. Average of Reducing sugars of the six experiments at 0.0, 0.2, 0.5, 1.0, 1.5, 2.0% H_2SO_4 was 26.74 mg/ml. Total phenolic sugars estimated in filtrate obtained after pretreatment of wheat straw at 121°C, 15 lb for 105 minutes were 5.54 mg/ml when pure water (0.0% H_2SO_4) was used, 9.07 mg/ml for 0.2% H_2SO_4 and maximum 10.34 mg/ml when 1.5% H_2SO_4 was used.

Batch-VIII: Analysis of Filtrate Obtained after Pretreatment of Wheat Straw at 121°C, 15lb for 120 min: Total sugars estimated were 2.89 mg/ml when pure water (0.0% H_2SO_4) was used. When dil. H_2SO_4 was used then total sugars were minimum 19.30 mg/ml at 0.2%

Table 6: Batch-VI; Analysis of filtrate obtained after pretreatment of 10g of wheat straw at 121°C, 15 lb for 90 min

Sr. NO.	Conc. of H_2SO_4 (%)	Dry Wt. of Residue (g)	Total sugars (mg/ml)	Reducing sugars (mg/ml)	Total phenols (mg/ml)
F_1	0.0	6.917	3.72	0.41	7.28
F_2	0.2	6.458	42.88	23.37	7.50
F_3	0.5	5.500	55.93	41.47	9.37
F_4	1.0	5.428	53.47	42.71	10.74
F_5	1.5	5.225	50.20	42.90	10.40
F_6	2.0	4.880	57.98	42.85	9.00

Table 7: Batch-VII; Analysis of filtrate obtained after pretreatment of 10g of wheat straw at 121°C, 15 lb for 105 min

Sr. No.	Conc. of H_2SO_4 (%)	Dry Wt. of Residue (g)	Total sugars (mg/ml)	Reducing sugars (mg/ml)	Total phenols (mg/ml)
F_1	0.0	7.531	0.59	03.05	5.54
F_2	0.2	6.585	16.73	26.65	9.07
F_3	0.5	6.205	35.17	04.90	10.27
F_4	1.0	5.752	28.87	42.37	10.47
F_5	1.5	5.719	48.11	42.39	10.34
F_6	2.0	5.710	31.10	41.08	9.87

Table 8: Batch-VIII; Analysis of filtrate obtained after pretreatment of 10g of wheat straw at 121°C, 15 lb for 120 min

Sr. NO.	Conc. of H_2SO_4 (%)	Dry Wt. of Residue (g)	Total sugars (mg/ml)	Reducing sugars (mg/ml)	Total phenols (mg/ml)
F_1	0.0	7.788	2.89	2.13	5.06
F_2	0.2	6.922	19.30	17.79	7.47
F_3	0.5	5.906	21.61	43.50	9.67
F_4	1.0	5.760	34.90	41.26	10.20
F_5	1.5	5.890	27.98	42.08	9.80
F_6	2.0	5.530	33.44	42.90	8.74

H_2SO_4 and maximum 34.90 mg/ml at 1.0% H_2SO_4. Average of total sugars of the six experiments at 0.0, 0.2, 0.5, 1.0, 1.5, 2.0% H_2SO_4 was 23.353 mg/ml (Table-8). Reducing sugars were 2.13 mg/ml when pure water (0.0% H_2SO_4) was used. But Reducing sugars were 17.79 mg/ml at 0.2% H_2SO_4 and maximum 43.50 mg/ml at 0.5% H_2SO_4. Total phenolic sugars estimated in filtrate obtained after pretreatment of wheat straw at 121°C, 15 lb for 120 minutes were 5.06 mg/ml, when pure water (0.0% H_2SO_4) was used, 07.47 mg/ml at 0.2% H_2SO_4 and maximum10.20 mg/ml when 1.0% H_2SO_4 was used.

Figure 2 presented the analysis of filtrate obtained from pretreatment of wheat straw from batch-V at 121°C and 75 min and showed that total phenol estimated in the filtrate were maximum (10.89 mg/ml) among all the batches and the results were promising. Under these conditions maximum amount of total sugars is 65.34 mg/ml and reducing sugars is 42.99 mg/ml at 1.5% H_2SO_4. Cara *et al.* [26] reported the maximum hemicelluloses recovery (83%) of olive tree biomass to be obtained at 170°C and 1.0% sulfuric acid. Sun and Cheng [27] pretreated rye straw and Bermuda grass for ethanol production at 121°C with different sulfuric acid conc. (0.6, 0.9, 1.2 and 1.5% w/w) and residence times (30, 60, 90 min). Saha *et al.* (2005) [15] evaluated the effects of both concentrated and dilute sulfuric acid pretreatment on wheat straw. Concentrated and dilute acid treatments yielded 49% and 63% of the total sugars content. Optimum acid conc. for maximum yield of carbohydrates in dilute acid treatment was 0.75% (v/v). The effect of temperature was also studied and formation of furfural was reported only at the highest temperature (180°C). Kootstra *et al.* (2009) [28] compared the efficiency of sulfuric acid with fumaric and maleic acids and concluded that maximu delignication occurred with dil. H_2SO_4 by virtue of which the yield of glucose after enzymatic hydrolysis reached to 98% and 96% for sulfuric acid and maleic acid respectively. Fumaric acid was found to be less effective than maleic acid for pretreatment of wheat straw.

The Fig. 3 presents the treatment of wheat straw with pure water (steam) without use of chemicals. The comparison of Total sugars, reducing sugar and Total phenols produced in flask F_1 of each of eight batches was also done and maximum production (mg/ml)

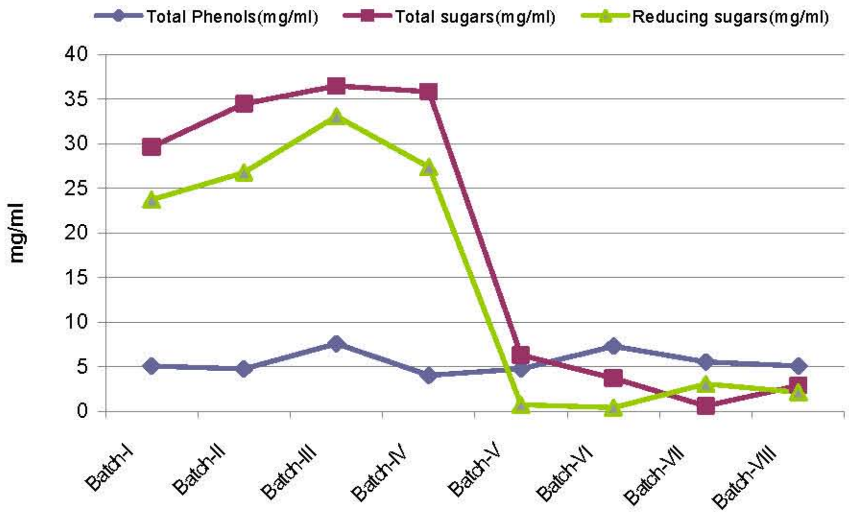

Fig. 3: Comparison of amounts of total phenols, total sugars and reducing sugars produced by pure water pretreatment (f1) in eight batches

of Total sugars (36.414), Reducing sugar (33.015) and Total phenol (7.58) was in Batch-III, (flask F_1), under pretreatment conditions 121°C, 15 lb for 45 min when pure water was used. Our results were supported by Malik *et al.* (2010) [29] who reported that steam (200°C) treated sugarcane bagasse produced maximum cellulase enzyme due to maximum delignification. In the next batches (IV-VIII) amount of total sugars, reducing sugars decreased sharply perhaps due to degradation and interactions during long time heating. Petersen *et al.* [30] did pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals. Linde M. [31] pretreated wheat straw with 0.2% sulfuric acid for ethanol production. Comparison between Fig 2 and Fig 3 shows that delignification was 43.66% more when pretreatment of wheat straw was done with 1.5% H_2SO_4 for 75 min than pure water for 45 min. Malik *et al.* (2010) [29] Used various chemicals Such as NaOH, H_2SO_4, H_2O_2 and $NaHClO_3$ and reported the maximum production of CMCase enzyme from sugar cane baggase pretreated with 1.5N sulfuric acid.

CONCLUSION

The conclusion of this research work is that best results of pretreatment of wheat straw depend upon a set of process parameters (Temperature, Autoclave time period and Concentration of acid). If temperature is increased then pretreatment time period is decreased and vice versa. Best conditions found in this batch wise study of the pretreatment of wheat straw were 121°C, 15 lb for 75 minutes when Concentration of H_2SO_4 was 1.5% (Batch-V, flask F_5). Under these condition the dry weight of residue obtained is minimum 5.009 g and weight loss is maximum (49.99%) due to maximum delignification (Table 5). It is concluded that filtrate obtained from pretreated wheat straw is rich of precious organic compounds like phenols and reducing sugars etc which can be separated, purified and used for manufacture of many drugs, chemicals, laboratory reagents and animal feed.

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