World Applied Sciences Journal 11 (12): 1524-1530, 2010
ISSN 1818-4952

Delignification of Wheat Straw with Acid and Hydro-Steam under Pressure

[1,3]*Zia-ullah Khokhar,* [2]*Q. Syed,* [2]*M. Nadeem,* [2]*S. Baig,* [2]*M. Irfan,* [1]*I. Gull,*
[1]*I. Tipu,* [1]*S. Aslam,* [1]*Zahoor Q. Samra and* [1]*M. Amin Athar*

[1]Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan
[2]Food and Biotechnology Research Center PCSIR Laboratories Complex,
Ferozpur Road Lahore, 54600 Pakistan
[3]Department of Chemistry, Government Islamia Post Graduate College Gujranwala, Pakistan

Abstract: This study was carried out to investigate the optimum conditions and concentration of sulfuric acid (H_2SO_4) used for pretreatment of wheat straw to achieve maximum delignification and exposure of cellulose for the production of fuel grade bioethanol. Pretreatment of wheat straw was conducted in eight batches at temperature 121°C and pressure 15 lb/inch^2 for different times varying15 to 120 minutes intervals separately. Each batch consisted of six 250 ml conical flasks, F_1, F_2, F_3, F_4, F_5 and F_6 and contained 10 g of wheat straw in each flask. 100 ml distilled water was added to the control flask F_1 while 100 ml of H_2SO_4 solution of different concentrations 0.2, 0.5, 1.0, 1.5 and 2.0% was added in flasks F_2-F_6 respectively. Residue obtained from each batch was used for the estimation of percentage of cellulose, lignin and delignification accordingly. The results showed that pretreatment of wheat straw with 1.5% H_2SO_4 (Flask F_5) of batch-V for 75 minutes resulted in 95% cellulose exposure/recovery. A 74% delignification with weight loss 49.9% was also observed in the same flask. On the other hand, pretreatment of wheat straw (control) with pure water (without H_2SO_4) at 121°C, 15 lb/inch^2 for 45 min (flask $F_{1,}$ Batch-III) showed 60% yield of cellulose and 57% delignification. Hence delignification (74%) of wheat straw with 1.5% H_2SO_4 for 75 min is higher than the delignification (57%) with pure water / without H_2SO_4 for 45 min pretreatment under the same condition.

Key words: Bioethanol · Pretreatment · Wheat straw · Delignification · H_2SO_4 · Plant biomass

INTRODUCTION

The increased prices, consumption and day to day decrease in supply of fossil fuels are the burning issue of world economy. Developing countries like Pakistan have been seriously affected and as a result inflation has been increased. This situation has put a pressure on scientific community to find the alternative renewable fuel. Most common renewable fuel today is bioethanol which is produced from lignocellulose of plant biomass [1]. Cheap and renewable agricultural byproduct such as rice and wheat straw, sugarcane bagasse and grasses are used for the production of bioethanol. Nowadays wheat straw is being considered a potential lignocellulosic raw material and is a likely candidate for use in second generation bioethanol production [2]. It is reported that the entire process of Bioethanol production involves four steps i.e. Pretreatment, Sccharification, Fermentation and Purification [3]. Pretreatment methods include physical, physicochemical, chemical (alkali, dilute acid treatment etc), biological, electrical, or a combination of these methods [4]. Among the physiochemical methods, a common pretreatment method is treatment with dilute sulfuric acid for disrupting the lignin-carbohydrate matrix, as well as to facilitate enzymatic cellulose hydrolysis [5-6]. The pretreatment process is necessary to remove the lignin and hydrolyze the polymers of cellulose into simpler unit for enzyme activity [7]. Pretreatment is the most expensive process in biomass-to-fuels conversion but it has great potential for improvements in ef?ciency and lowering of costs through further research and development [8].

Corresponding Author: Zia-ullah Khokhar, Institute of Biochemistry and Biotechnology, Punjab University, Lahore, Pakistan, Department of Chemistry, Government Islamia Post Graduate College Gujranwala, Pakistan. Tel # 0092-300-7432748.

Pretreatment of wheat straw with dilute H_2SO_4 is an effective and promising technique at commercial level because ethanol percentage production is high when compared with treatment with concentrated H_2SO_4. The concentrated acid treatment has drawbacks such as higher energy consumption, equipment corrosion and longer reaction time as well as obligation for acid recovery after treatment that largely limits its application [9]. While dilute acid treatment e.g. 0.5% and 1% H_2SO_4 along with high temperatures is most suitable and economical. Low acid concentration, reaction time and temperature are three parameters which can be further optimized and set to economical conditions. [9, 10]. The diverse advantages and disadvantages are associated with each pretreatment method. The pretreatment of wheat straw with dilute and concentrated sulfuric acid and reported that optimum acid dose (0.75v/v) and high temperature (180°C) is suitable for maximum yield of carbohydrates and for the formation of furfural [11].

In this study we report the optimization of process variables (concentration and residence time) for pretreatment of wheat straw in batch-wise experiments for production of bioethanol. The development of the production of bioethanol will strengthen the technological base of country in addition to saving foreign exchange that Pakistan spends on import of fossil fuel.

MATERIAL AND METHODS

Preparation of Lignocellulosic Biomass: Wheat straw (Lignocellulosic biomass) was obtained from wheat (*Triticum aestivum L.*) Crop-2010 grown at Fazal Agricultural Form at Mareywala khoh village Sohian District Gujranwala, Pakistan. Wheat straw was washed to remove the undesired particles and dried. The wheat straw was ground into powder form (1-2 mm) with hammer beater mill.

Pretreatment: Pretreatment of wheat straw was carried out in eight batches(I to VIII) at 121°C, 15 lb/inch^2 for varying time intervals (15, 30, 45, 60, 75, 90, 105 and 120 min). In each batch 10 gm 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 was added while in flasks F_2-F_6 100 ml of dilute H_2SO_4 of different Concentrations (0.2, 0.5, 1.0, 1.5 and 2.0% respectively) was added. Six flasks of each batch were autoclaved at 121°C, 15 lb/inch^2 for respective time i.e. 15, 30, 45, 60, 75, 90, 105 and 120 min. After treating the flasks at high temperature and pressure, slurry was filtered. The filtrate was analyzed for total sugars, reducing sugars and total phenols [12]. The residue was packed in muslin cloth bags of small size (6.0 cm X 8.0 cm) and washed for 4 to 5 times using distilled water till neutral pH reached and then dried in oven. These oven dried samples were packed in labeled plastic zip lock-bags for further use [13].

Cellulose Content Estimation: Cellulose contents in the untreated and treated wheat straw were estimated by taking one gram of 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 samples and heated on electric reflux for 30 minutes. The residue was diluted, filtered and washed with distilled water. The residue was taken in the crucible and then dried in oven at 105°C for 14 hours. Weighed (W_2) the dried sample and added concentrated HNO_3 to wet the sample's residue for charring and placed it in the Muffle furnace at 550 °C for 4 hours. Calculate the total weight of the ash [14]. Cellulose (% age) was calculated using the following formula.

$$\text{Percentage of cellulose} = \frac{w_2 - w_3}{w_1} \times 100$$

Lignin Content Estimation: The percentage of lignin content in untreated and treated samples was measured by considering lignin as remaining solid residue after hydrolysis with 1.25% H_2SO_4 for two hours and hydrolysis with 72% H_2SO_4 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. The lignin and delignification percent was expressed by using the formulae given below described by Milagres, 1994 [15].

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

$$\text{Delignification (\%)} = \frac{\text{Initial Lignin content} - \text{Lignin in Pretreated sample}}{\text{Initial lignin content in Sample}} \times 100$$

Ash Estimation: Ash of the wheat straw was calculated according to the standard method as described in AOAC [16]

Moisture Estimation: Moisture of wheat straw was determined by gravimetric method as described [17].

RESULTS AND DISCUSSION

Proximate analysis of untreated wheat straw is represented in Pie chart (Fig.1). It was found that wheat straw of Pakistani variety contains (% w/w): Cellulose 36.0, hemicellulose 27.9, lignin 22.0, moisture 7.8, protein 3.1 and ash 3.2. It is established that wheat straw consists of mainly three components cellulose, hemicellulose and lignin. It is described that cellulose, hemicellulose and Lignin content of wheat straw are in the range of 33 to 40, 20 to 25 and 15 to 20 (% w/w), respectively[18]. Saha *et al.* [11] reported that 27.7% Hemicellulose and crude protein 3.48% (w/w) was found in wheat straw.

The batch wise analysis of residue obtained after pretreatment of wheat straw at 121°C, 15 lb/inch^2 for different time period (15, 30, 45, 60, 75, 90, 105 and 120 min) is shown in table 1. In Batch-I, after pretreatment of wheat straw at 121°C, 15 lb/inch^2 for 15 min, the maximum cellulose (59%) was estimated in Flask F_5 and minimum cellulose (46%) in the flask F_2. It was also observed that maximum delignification (68.64%) occurred with 1.0 % H_2SO_4 while minimum delignification (41%) was observed with pure water (F_1). In Batch-II preparation, maximum cellulose (73%) and minimum cellulose (52%) was obtained with 2.0% and 0.2% H_2SO_4 respectively and delignification (70%) was found with 1.5% H_2SO_4. In Batch-III preparation, maximum (61%) and minimum (46%) cellulose yield was found by treatment with 1.5% and 0.2% H_2SO_4 respectively. In this batch, a good yield of cellulose (60%) was estimated by treating with pure water (F_1) which is highest among all the batches and maximum delignification (71.8%) was found with 1.0% H_2SO_4.

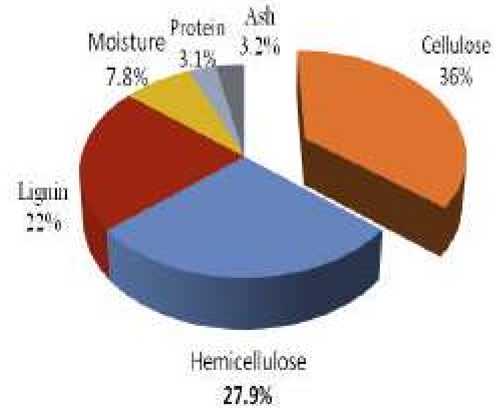

Fig. 1: A Pie chart for the proximate analysis of untreated wheat straw.

In Batch-IV, maximum cellulose (61%) and minimum cellulose (47%) was yielded with 1.0% and 0.2% H_2SO_4 respectively while maximum delignification (63.2%) was observed with 0.5% H_2SO_4. In Batch-V, best result was achieved and the optimum pretreatment conditions were 121°C, 15 lb/inch^2 pressure, 75 minutes and 1.5% H_2SO_4. Under these conditions it was observed that percentage of cellulose in wheat straw was increased up to 95% and lignin was decreased by 74.2%. In this study the method of delignification (74.2%) of wheat straw is better than the reported method [19]. It is reported that 70.70 % delignification from sugarcane bagasse was observed with 2.5% KOH at 121 °C, 15 lb/inch^2 pressure, 45 minutes [19]. Our results of cellulose recovery (95%) are relatively better than Petersen *et al.*, who recovered 93-94% cellulose by treatment of wheat straw at high temperature

Table 1 Batch wise analysis of residue obtained after pretreatment of wheat straw at 121°C, 15 lb for 15 to 120 min.

Flask NO.	Conc. Of H_2SO_4 (%)	Batch-I, (15 min) Cellulose%	Lignin %	Delignification %	Batch-II, (30 min) Cellulose %	Lignin%	Delignification %	Batch-III, (45 min) Cellulose %	Lignin %	Delignification %	Batch-IV, (60 min) Cellulose %	Lignin %	Delignification %
F1	0.0	52	13	41	13	41	60	9.5	57	55	14	36	53
F2	0.2	46	10	55	52	10	55	46	9.7	56	10	55	47
F3	0.5	53	8.5	61	66	8.1	65	52	7.7	65	54	8.1	65
F4	1.0	52	6.9	69	69	6.9	69	54	6.2	72	61	6.2	72
F5	1.5	59	8.4	62	53	6.6	70	61	7.1	68	57	6.3	71
F6	2.0	52	8.2	64	73	6.9	69	54	8.1	65	58	6.8	69

Flask NO.	Conc. Of H_2SO_4(%)	Batch-V (75 min) Cellulose%	Lignin %	Delignification %	Batch-VI (90 min) Cellulose %	Lignin%	Delignification %	Batch-VII (105 min) Cellulose %	Lignin %	Delignification %	Batch-VIII (120 min) Cellulose %	Lignin %	Delignification %
F1	0.0	53	13	41	54	11	50	45	13	41	49	13	41
F2	0.2	63	9.9	56	62	9.5	57	57	7.9	64	56	10	55
F3	0.5	79	6.9	69	62	9.1	59	61	6.7	69	61	7.1	68
F4	1.0	81	7	70	63	6.2	72	59	6.9	68	70	6.7	69
F5	1.5	95	5.9	74	65	6.3	71	63	6.8	69	66	7.5	66
F6	2.0	47	6.4	71	47	9.1	59	69	7.2	67	64	8.2	63

Note; (i) In F_5 of Batch-V; Maximum cellulose (95%), Minimum Lignin (5.9%) and Maximum delignification (74%) was estimated among all the eight batches.

(ii) Amounts of Cellulose, Lignin and Delignification are rounded to nearest whole Numbers.

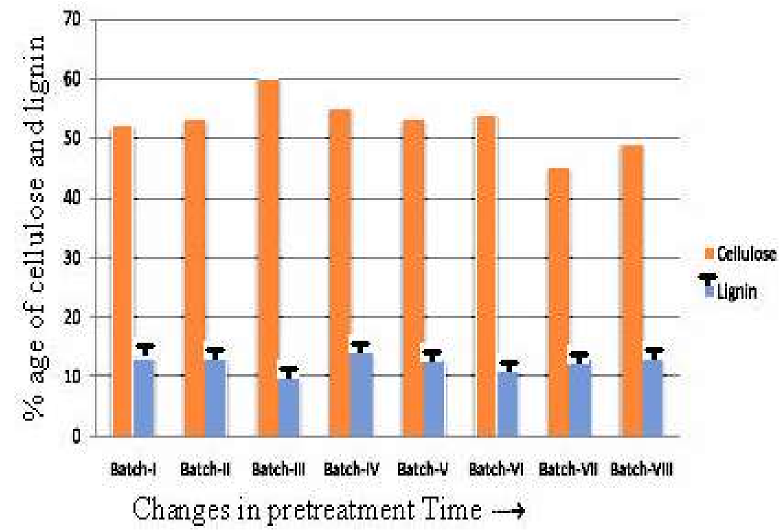

Fig. 2: Comparison of cellulose and lignin estimated in residue obtained after pretreatment with pure water (F_1) in eight batches

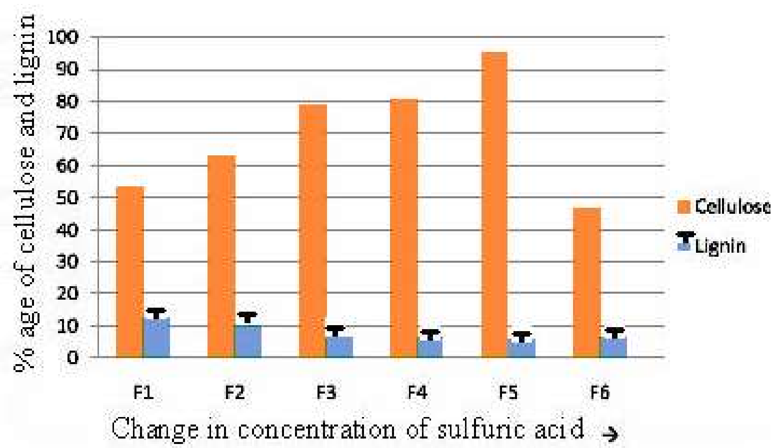

Fig. 3: Comparison of amounts of cellulose and lignin estimated in residue of wheat straw in Batch-V

(195°C) for 6-12 minutes [20]. The results achieved in this laboratory are better due to use of low concentration of H_2SO_4 (1.5%) at comparatively low temperature (121°C) which also results in maximum delignification (74%) of wheat straw. In Batch-VI, the recovery of cellulose was between 47 % to 65 % and maximum delignification was 71.8%. The ash content ranged between 1.2 % to 2.6 %. In Batch-VII, 45% and 69 % cellulose was recovered without and with 2.0% H_2SO_4, respectively. Delignification was 41% and 71% without and with 0.5% H_2SO_4 respectively. Highest amount of ash (3.6%) was noted in flask F_2. In Batch-VIII, cellulose, lignin and delignification ranged between 49-70%, 7.1-13% and 41-69% respectively. The results were observed excelent in batch-VIII due to long autoclave time (120 minutes) but the conditions are not economical.

Percentage comparison of cellulose and lignin in residue obtained after pretreatment with pure water (F_1) in all the eight batches is shown in Fig 2. Percentage of cellulose in wheat straw was increased from 36% to 60% after pretreatment with pure water (without H_2SO_4), 121°C and 15lb /inch^2 and 45 minutes autoclave time. Our results were different and less cellulose was recovered (60% at 121°C) than the procedure of Petersen *et al*., who recovered more cellulose (93-94%) at 195°C and this may be due to prominent difference in temperature [20]. Yang and Wyman reported that less lignin was removed with HCl as pretreatment than with hot water only and sulfuric acid and acetic acid only removed 12% and 37% of the lignin respectively [21].

The percentage comparison of cellulose and lignin estimated in the residue of batch-V obtained after pretreatment of wheat straw at 121°C, 15 lb/inch^2 for

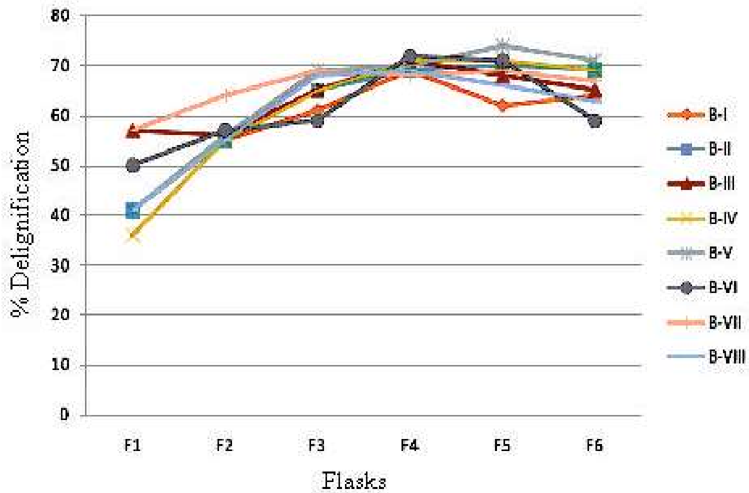

Fig. 4: Comparison of delignification (%) of wheat straw in eight batches.

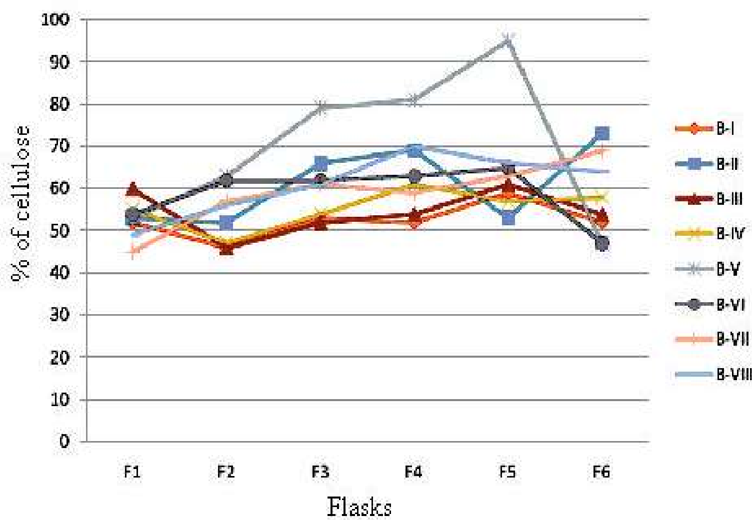

Fig. 5: Comparison of cellulose percentage in residue obtained after pretreatment of wheat straw in eight batches.

75 minutes is shown in Fig 3. The % age of cellulose increased in flask F_1 to F_5 (53, 63, 79, 81 and 95%, respectively) and then suddenly decreased in flask F_6 (47%). This decrease may be due to hydrolysis of cellulose to glucose. Maximum Percentage of Cellulose (95%) was obtained in flask F_5 under pretreatment conditions of 121°C, 15 lb/inch^2 pressure, 75 minutes time and 1.5% H_2SO_4 and under same conditions delignification was also maximum (74.%) in the same flask $F_{5.}$ Pedersen *et al.,* reported that at pH 13, 68% acid insoluble lignin was removed from the solid fraction of wheat straw [22]. Taherzade explained that dilute-acid hydrolysis is probably the most commonly applied method among the chemical pretreatment methods [23]. Dilute acid method is equally effective both for pretreatment of lignocelluloses and hydrolysis of cellulose into glucose. According to Wyman, almost 100% hemicellulose removal is possible by dilute-acid pretreatment but lignin is not completely removed by dilute acid pretreatment [24]. It only disrupts lignin, decrease the crystalinty and increase the susceptibility of cellulose for enzymatic attack.

The comparison of percentage delignification after Batch-wise pretreatment of wheat straw is shown in Fig. 4. It showed a great variation in the percentage delignification (36% to74%) among all the flasks and batches. Thus maximum delignification was observed at 75 minutes after steaming process, 15 lb/inch^2 pressure, 121°C temperature and treatment with 1.5% $H_2SO_{4.}$ It is also shown in Fig. 3 that narrow variation in delignification (68-72%) was in flask $F_{4,}$ treated with 1.0 % $H_2SO_{4,}$ hence no significant effect of change of steaming time is observed under these conditions.

The comparison of the cellulose percentage in the residue obtained after pretreatment of wheat straw in eight batches is shown in Fig. 5. It shows that maximum cellulose percentage was observed in almost all the flasks of batch-V indicating that 75 minutes pretreatment time is the best time period for delignification of wheat straw. It is noted that highest amount of cellulose (95%) was obtained with 1.5 % H_2SO_4 treatment but sudden decrease (47%) of cellulose in the Flask F_6 and this may be due to increased degradation of cellulose into glucose with 2.0% H_2SO_4. Saha *et al*., stated that dilute acid treatment removes the hemicelluloses successfully and decreases the need of use of hemicelluase enzyme for degradation of biomass [11]. Cara *et al*., reported that maximum hemicelluloses recovery (83%) of olive tree biomass was obtained at 170°C and 1.0% sulfuric acid [25]. Sun and Cheng (2005) pretreated the rye straw and Bermuda grass for ethanol production at 121°C with different concentrations of H_2SO_4 (0.6, 0.9, 1.2 and 1.5% w/w) for 30, 60, 90 minutes respectively [13]. Saha *et al*., [11] described the effects of both concentrated and dilute sulfuric acid treatment on wheat straw. Concentrated and dilute acid treatments yielded 49% and 63% of the total sugars content. Optimum acid concentration for maximum yield of carbohydrates in dilute acid treatment was 0.75% (v/v) and formation of furfural was reported only at the higher temperature (180°C).

Zhu *et al.* [26] reported that during hot acid pretreatment, some of polysaccharides (hemicelluloses) are hydrolyzed and resulting free sugars degraded to furfural (from pentoses) and to 5-hydroxymethylfurfural (HMF). Qian *et al*., claimed that furfural and HMF inhibit the yeast cell's growth rate which in turn effect on ethanol production rate and ethanol yield [27]. On the other hand the production HMF means loss of fermentable sugars. Kootstra *et al*., compared the efficiency of sulfuric acid with fumaric and maleic acids for delignification and it was observed that maximum delignification 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 [28]. Fumaric acid was found less effective than maleic acid for pretreatment of wheat straw. Rebecca *et al*., studied and compared the effectiveness of sulfuric acid pretreatments for conversion of cotton stalks to ethanol and reported that 2% acid treatment at 121°C for 90 minutes and 15 lb/inch^2 were optimal pretreatment parameters [29]. The results of this study are better than Rebecca because 95% cellulose is recovered at 1.5% acid treatment for 75minutes at 121°C and 15 lb/inch^2. In this study the maximum delignification (74%) and recovery of cellulose (95%) is obtained by pretreatment of wheat straw with dilute H_2SO_4.

CONCLUSION

Lignocellulosic agricultural by-products (wheat straw, rice straw, grasses and sugarcane baggase etc) are easily available at low price which can be used for bioethanol production. In this study we have optimized the pretreatment parameters for maximum delignification (74%) and high yield of cellulose (95%) recovery from wheat straw to produce fuel grade Bioethanol. Pretreatment of plant biomass (wheat straw) with dilute sulfuric acid is a promising process for delignification and cellulose recovery which requires less concentration of sulfuric acid foe shorter time period.

ACKNOWLEDGEMENTS

The financial support was provided by Ministry of Science and Technology (MoST), Govt. of Pakistan through Project of "Production of Bioenergy from Plant Biomass". Author acknowledges the scholarship from the Higher Education Commission (HEC), Govt. of Pakistan under the Indeginous 5000 fellowship program. Authors are thankful to laboratory staff of IBB, Punjab University, Lahore and FBRC, PCSIR Lahore, Pakistan.

REFERENCES

1. Hahn-Hagerdal, B., M. Galbe, M.F. Gorwa-Grauslund, G. Liden and G. Zacchi, 2006. Bio-ethanol-the fuel of tomorrow from the residues of today. Trends Biotechnol., 24: 549-556.
2. FAOSTAT, Wheat production quantity EU 27, Food and Agriculture Organization of the United Nations, 2007.
3. Margeot, A. Hahn-Hagerdal, M. Edlund, R. Slade and F. Monot, 2009. New improvements for lignocellulosic ethanol. Curr. Opin. Biotechnol., 20: 372-380.
4. Mosier, N.S., C. Wyman, B. Dale, R. Elander and Y. Lee, 2005. Holtzapple, M. Ladisch, M.R. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol., 96: 673-686.

5. Baig, M.M.V., M.L.B. Baig, M.I.A. Baig and M. Yasmeen, 2004. Saccharification of banana agrowaste by cellulolytic enzymes. Afr. J. Biotechnol., 3: 447-450.
6. Yang, B. and C.E. Wyman, 2008. Pretreatment: the key to unlocking low-cost cellulosic ethanol, Biofuels Bioproduction Biore?ning, 2: 26-40.
7. Klinke, A.B. Thomsen and B.K. Ahring, 2004. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass, Appl. Microbiol. Biotechnol., 66: 10-26.
8. Hsu, T.A., M.R. Ladisch and G.T. Tsao, 1980. Alcohol from cellulose, Chemical Technol., 10(5): 315-319.
9. Hendriks, A. And G. Zeeman, 2009. Pretreatment to enhance the digestibility of lignocellulosic biomass. Bioresour, Technol., 100: 10-18.
10. Sun, X.F., R.C. Sun, Y. Su and J.X. Sun, 2004. Comparative study of crude and purified cellulose from wheat straw, Jr. Agricult. Food Chemist., 52: 839-847.
11. Saha, B.C., L.B. Iten, M.A. Cotta and Y.V. Wu, 2005. Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. Process Biochemist, 40: 3693-3700
12. Khokhar, U.Z., Q. Syed, S. Baig, M. Nadeem, M. Irfan and A.M. Athar, 2010. Study on Filtrate Obtained from Batch-Wise Pretreatment of Wheat Straw at Different Concentrations of H_2SO_4 and autoclave Time. World Appl. Sci. Jr., 10(9): 1047-1055.
13. Sun, Y. And J.J. Cheng, 2005. Dilute acid pretreatment of rye straw and Bermuda grass for ethanol production. Bioresource Technol., 96: 1599-606.
14. Gopal, K. and S.K. Ranjhan, 1980. laboratory manual for nutrition research. Roland press (India) Private Ltd. New Delhi.
15. Milagres, A.M.F., 1994. Producao de xilanases por penicillium janthinellum aplicacao das enzimas no branqueamento de polpas kraft. Campinas 137p. Tese (Doutorado)-Universidade Estadual de Campinas.
16. Association of Official Analytical Chemists (AOAC), 2005. Official methods of analysis, 14th edition, Dc.
17. Blakeman, J.P., A.R. McCracken and D.A. Seady, 1988. Changes braught about in solid substrates after fermentation of mixtures of cereals and pulses with oryaze. Jr. Sci. Food Agricult., 45: 109-118.
18. Prasad, S., A. Singh and H.C. Joshi, 2007. Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resour. Conser. Recycle, 50: 1-39.
19. Irfan, M., S. Abbas, S. Baig, M. Gulsher, M. Nadeem and Q. Syed, 2010. Pretreatment: A potential technique to enhance the enzymatic hydrolysis. Word Jr. Agricult. Sci., 6(4): 440-445.
20. Petersen, M.O., J. Larsen and M.H. Thomsen, 2009. Optimization of hydrothermal pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals. Biomass Bioengineer, 33: 834-840.
21. Yang, B. And C.E. Wyman, 2004. Effect of xylan and lignin removal by batch and flowthrough pretreatment on the enzymatic digestibility of corn Stover cellulose. Biotechnol. Bioengineer, 86: 88-95.
22. Pederson, M., A. Viksø-Nielsen and S.A. Meyer, 2010. Monosaccharide yields and lignin removal from wheat straw in response to catalyst type and pH during mild thermal pretreatment, Process Biochemist., 45(7): 1181-1186.
23. Taherzadeh, M.J. and K. Karimi, 2007. Acid-based hydrolysis processes for ethanol from lignocellulosic materials: a review. Bioresour., 2: 472-499.
24. Wyman, C.E., 1996. Handbook on bioethanol, Production and utilization, Taylor and Francis.
25. Cara, C., E. Ruiz, J.M. Oliva, F. Saez and E. Castro, 2007. Conversion of Olive tree biomass into fermentable sugars by dilute acid pretreatment and enzymatic saccharification, Bioresour. Technol., 99: 1869-1876.
26. Zhu, Z. And N. Sathitsuksanoh, T. Vinzant and J.D. Schell, D.J. McMillan and P.H.Y. Zhang, 2009. Comparative study of corn stover pretreated by dilute acid and cellulose solvent-based lignocelluloses fractionation: enzymatic hydrolysis, supramolecular structure and substrate accessibility, Biotechnol., Bioengineer, 10: 1002/bit. 22307.
27. Qian, M.R., M. Nimlos, D.K. Davis and Johnson, M.E. Himmel, 2005. A binitio molecular dynamic simulations of beta-d-glucose and beta-d-xylose degradation mechanisms in acidic aqueous solution, Carbohydrate Res., 340: 2319-2327.
28. Kootstra, A.M.J., H.H. Beeftink, E.L. Scott and J.P.M. Sanders, 2009. Comparison of dil. mineral and organic acids pretreatment for enzymatic hydrolysis of wheat straw. Biochemist. Engineer. Jr., 46: 126-131.
29. Rebecca, A., Silverstein, Ye Chen, R. Ratna, Sharma-Shivappa, Michael D. Boyette and J. Osborne, 2007. A comparison of chemical pretreatment methods for improving sacchari?cation of cotton stalks, Bioresour. Technol., 98: 3000-301.