

Pretreatment of Lignocellulosic Material - Bagasse for Ethanol Production

Sushma Kumari¹ and Dr. Rakesh Kumar²

Research Scholar, Department of Chemistry¹

Assistant Professor, Department of Chemistry²

Shri J.J.T. University, Jhunjhunu, Rajasthan, India

Abstract: Lignocelluloses are most viable raw materials which can be utilized for the fermentative production of ethanol. However, to convert them for biodegradation / hydrolysis these need to be pretreated by various methods. In the present study, biodegradability / digestibility of bagasse has been identified using different physical and chemical methods. The reagents used for pretreatment of bagasse are NaOH, H₂SO₄ and HCl, whereas grinding, milling, steam explosion have been used as physical methods. A significant removal of lignin has been obtained in all the methods used, which ranged from 32-85%. Alkali treatment and steam explosion gave best results, but hydrochloric acid was comparatively less effective and required more time and drastic experimental conditions. Sulphuric acid too was effective in removing lignin and increasing the digestibility of cellulose.

Keywords: Lignocellulosic waste, Bagasse, Pretreatment, Ethanol etc.

I. INTRODUCTION

Fossil fuels including crude oil have been the major resource to meet global energy demand but these are continuously diminishing and at the same time prices are increasing at a fast pace. At present global annual demand of petroleum products is 17000 million metric tons.

Indian requirement of petroleum products is 120 MMT out of which major portion is imported at the cost of heavy outflow of foreign exchange. Ethanol is viewed as an alternate fuel which could partially or fully replace gasoline. Use of ethanol made from lignocellulosic biomass as a renewable transportation fuel could potentially offer many benefits.¹ Compared to fossil fuels, the bio-ethanol contribution to the net emission of CO₂ is low². Blended with gasoline, combustion of ethanol in car engines lower the emissions of many pollutants such as CO, hydrocarbons and volatile organic compounds³. Further more, ethanol increases the octane number⁴.

The lignocellulosics comprised of cellulose and hemicellulose bound together by a third component lignin can be utilised to produce ethanol⁵. From the point of view of ethanol fermentation, they are hard to work with two reasons⁶. First, the lignin protects the cellulose and hemicellulose from the attack by enzymes⁷. Second, when do manage to reach the cellulose and hemicellulose they are hindered by the crystalline structure of these molecules⁸.

The low degradation rate, as well as low extent of conversion of native, untreated lignocellulosic inhibits the development of an economically feasible hydrolysis process. Several pretreatment methods are used which increase of porosity, reduction of cellulose crystallinity, removal of lignin and hemicelluloses⁹, to make the carbohydrates available for further transformation process¹⁰.

In the present study, different physical and chemical pretreatment methods have been adopted for delignification of bagasse.

II. MATERIALS AND METHODS

Raw material (Substrate)

The bagasse obtained from Modi Sugar Factory, Modinagar. The bagasse was washed thoroughly for removal of sugars from bagasse fiber, dried under sun and further in oven.

Pretreatment Methods

The pretreatment of bagasse was done using different physical (grinding, milling and steam explosion) and chemical (NaOH, H₂SO₄ and HCl) methods.

Grinding

The oven dried (90°C) bagasse was cooled and homogenized in an electric blender machine at 5000 rpm for ten minutes at intervals of 2 min. each. The finely powdered bagasse was then sieved to 35, 65 and 85 mesh sizes using Tyler sieves. The grinding at above rpm breaks the lignin-cellulose complex present in bagasse. The powdered bagasse was analysed for decrease in lignin content.

Milling

Dried bagasse was cut into small pieces and then milled through milling machine for 3-4 times at time interval of 5 to 10 minutes each. This milled bagasse was analysed for lignin, hemicellulose and cellulose to assess decrease in lignin content.

Steam Explosion

In this method, dried bagasse kept in an autoclave 121°C, 15 psig of steam for 15, 30 and 45 minutes without adding any chemical. The swelled bagasse after treatment with steam was analysed for any change in its constituents including lignin.

Alkali (NaOH) Pretreatment

NaOH of different concentrations (4, 6, 8 & respectively) was used for different periods of time. For such treatment, 10 g of bagasse was mixed with 100 ml solution of various concentration of NaOH at 40°C for a period of 5, 10, 20, 40, 90, 120 and 140 minutes after which it was washed with water thoroughly, dried and analysed for various constituents.

Acid Pretreatment

The sulphuric acid of different concentrations ranging from 1 to 10% was used at 40°C for a period of 20 minutes, 2 hours and 20 hours. The hydrochloric acid at concentrations of 3, 6 and 9 % was used at 90°C for a period of 30, 60 and 120 minutes. For these studies 10 g bagasse was mixed with 100 ml of acid solution of different concentrations and kept at 90°C for required period of time.

In general, for all the pretreatment methods, 10 g dried bagasse was pretreated by different agents, washed thoroughly with water, dried and digested with 1% NaOH for 1 hour for extraction of lignin. The residual bagasse was analysed for hemicellulose and cellulose content. All the experiments were done in triplicate and the matching values are reported.

Analytical Methods

Moisture was determined by drying bagasse in an oven 90°C over night and observing the change in its weight after cooling. The procedure was repeated to a constant weight.

For the extraction of lignin, dried sample (10g) was mixed with 1% NaOH (180ml) and then digested in an autoclave at 121°C for 1 hour. The digested bagasse was washed with distilled water and washings were used for lignin precipitation by 5N HCl. The precipitated lignin was filtered and the filtrate as well as residual bagasse was used for analysis of hemicellulose and cellulose. The precipitated lignin was estimated by Denis reagent method 11.

Hemicellulose content was determined by taking residual bagasse in 10 ml of acetic-nitric acid reagent and refluxed for 30 minutes on a water bath, then cooled and filtered. The filtrate was used for hemicellulose estimation by Orcinol reagent method 12.

0.2 g of residual bagasse left after hemicellulose extraction was dissolved in 4 ml of 67% H₂SO₄ at room temperature for 30 minutes and used for the analysis of cellulose by Anthrone reagent method 13.

III. RESULTS AND DISCUSSION

The lignocellulosic substrates contain lignin which acts as a cementing material for cellulose. This strong bond needs to be softened so as to free the cellulose for any enzymatic, microbial or chemical utilisation. This was done through physical, chemical methods which help free part of lignin or soften the bond between cellulose, hemicelluloses and lignin in lignocellulosic since higher the quantity of lignin in any substrate, the more it will be resistant towards any kind of degradation. The pretreatment of bagasse by grinding, milling, steam explosion, alkali and acids have been presented in Tables-1 to 6

The analysis of bagasse of different sizes showed an increase in the cellulose content of powdered bagasse as the mesh size increased. The maximum value of cellulose contents (42.82%) was obtained with mesh size of 85 against a control value of 41.28% (Table 1). However, hemicelluloses content showed a reverse trend and the content of hemicelluloses decreased with increase in mesh size. The lignin content in the ground bagasse showed continuous decrease and the values were 22.90, 21.70 and 18.30% in bagasse of size 35, 65 and 85 mesh respectively against a control value of 26.98%. Thus lignin removal varied from 14.67% to 32.17%, the maximum being with 85 mesh size bagasse powder. This showed that lignin removal is more with smaller bagasse particles due to larger surface area to volume ratio thus making it more accessible for further utilisation.

In this study bagasse was milled at time intervals of 15, 35 and 75 minutes, cooled and analysed for cellulose, hemicellulose and lignin content. The results similar to those with grinding were obtained. Here too, hemicellulose and lignin decreased from 8.84 to 6.24% and 26.06 to 16% respectively causing lignin removal to an extent of 40.6% in 75 minutes (Table 2). This decrease in lignin content is attributed to compressive force of milling causing a decrease in degree of polymerization in the structure of lignin and thus reducing the cohesive force between lignin and cellulose.

In steam explosion, bagasse was steamed in an autoclave at 121°C at a pressure of 15 psig for 15, 30, 45 minutes. This pretreatment remarkably increased cellulose content to 50.90% from a control value of 41.28% and a considerable decrease in hemicellulose and lignin content was observed with maximum lignin decomposition of 57.38% when bagasse was treated under those conditions for 45 minutes (Table 3). Steam explosion treatment removes lignin to a large extent as a result of swelling of bagasse which becomes more of a pulpy product from which lignin and cellulose are easily extractable. Hence, steam explosion of bagasse is a very effective pretreatment method and it provides conducive environment for microbial/enzymatic reaction and/or cellulose hydrolysis.

Alkali treatment of bagasse resulted in increased cellulose content and significant decrease in lignin content at all the concentrations and at all time intervals (Table-4). The exposure of bagasse to higher concentrations of alkali and for longer periods of time provided still better results. The cellulose content in treated bagasse was treated with 10% NaOH for a period of 140 minutes. However, it is seen from the Table-4 that the alkali treatment is very effective method for removal of lignin from bagasse the lignin removal was as high as 84.58% obtained by using 8% alkali for 140 minutes, which is higher than reported earlier¹⁴. At other time intervals too, lignin removal was significant (over 67% in 40 minutes) at all concentration of alkali.

It was observed that treatment of bagasse by all alkalis for longer duration at nearly all concentrations. This may be attributed to the fact that the presence of alkali for a long period in bagasse causes better swelling of bagasse resulting into loosening/softening of cellulose-lignin matrix, leaving the lignin which consequently gets dissolved in alkali. During delignification, the relative cellulose content increases but hemicellulose content decreases due to decrease in hemicelluloses content in case of all pretreatments done.

Treatment with 1% sulphuric acid is more effective in removing lignin than 10% sulphuric acid especially when bagasse was treated duration of 20 and 180 minutes. The treatment by 10% sulphuric acid even for 1200 minutes did not improve delignification. Mild sulphuric acid has been used by several workers for the pretreatment of lignocellulosics¹⁵.

The pretreatment of bagasse with hydrochloric acid at 3, 6 and 8% concentrations has been done at 90°C for 30, 60 and 120 minutes respectively. The pattern of results was similar to those obtained with H₂SO₄. The cellulose content increased while lignin and hemicellulose content of bagasse decreased (Table 6). However the extent of lignin removal by HCl was on the lower side. The highest removal of lignin (59.71%) was seen at 6% concentration of HCl at a treatment time of 120 min. 8% HCl treatment also produced similar results.

The data given above show that bagasse, like other lignocellulosics, can be effectively pretreated by various physical and chemical means for the removal of lignin and to increase the digestibility of cellulose. Among the above methods, steam explosion and alkali treatment gave the best result. Milling and grinding also resulted in partial removal of lignin from bagasse. Nearly similar results were obtained in case of acid treatment. The H₂SO₄ treatment was found to be simple as it could be done at a temperature of 40°C while hydrochloric acid treatment was effective at higher temperatures only.

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Table 1: Delignification of Bagasse by Grinding.

Mesh size	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Percent Reduction in Lignin Content
Control	41.28	25.74	26.98	-
35	41.80	15.94	22.90	14.67
65	35.60	24.94	21.70	19.57
85	42.82	18.84	18.30	32.17

Table 2: Delignification of Bagasse by Milling.

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Time* (min)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Percent Reduction in Lignin Content
Control	41.28	25.74	26.98	-
15	44.80	8.84	26.06	3.40
35	46.86	7.14	18.70	36.38
75	50.10	6.24	16.00	40.60

Table 3: Delignification of Bagasse by Steam Explosion in Autoclave at 121°C.

Time* (min)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Percent Reduction in Lignin Content
Control	41.28	25.74	26.98	---
15	50.90	19.94	17.52	35.10
30	49.80	19.14	13.50	50.00
45	47.40	16.74	11.72	57.38

Table 5: Delignification of Bagasse with Different Concentration of H₂SO₄ at 40°C.

Time* (min)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Percent Reduction in Lignin Content
Control	41.28	25.74	26.98	---
1 %				
20	48.60	9.24	12.70	52.92
120	49.70	12.94	12.40	54.40
1200	38.40	5.94	12.50	53.66
10 %				
20	41.30	10.54	14.90	44.77
12	45.60	14.21	16.60	38.47
1200	40.10	9.74	16.88	37.43

Table 6: Delignification of Bagasse with Different Concentration of HCl at 40°C.

Time* (min)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Percent Reduction in Lignin Content
Control	41.28	25.74	26.98	-
3 %				
30	35.66	8.76	21.20	21.42
60	36.68	5.89	19.40	28.09
120	42.98	4.48	16.20	39.95
6 %				
30	41.16	10.86	18.61	31.02
60	43.89	9.20	17.77	34.13
120	46.44	6.89	10.87	59.71
8 %				
30	45.01	10.68	19.58	25.94
60	45.46	8.55	25.94	30.02
120	45.90	5.89	14.16	47.51

Table 4: Delignification of Bagasse with Different Concentration of NaOH.

Time* (min)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Percent Reduction in Lignin Content
4 % NaOH				
Control	41.28	25.74	26.98	-
5	37.62	18.00	20.24	24.61
10	39.80	13.64	15.10	44.03
20	40.55	10.38	10.72	60.26
40	42.86	8.76	9.24	65.75
90	46.38	8.22	8.80	67.38
120	46.98	7.78	8.37	68.97
140	48.40	7.34	7.90	70.71
6 % NaOH				
5	39.20	9.36	20.00	25.87
10	44.00	8.30	15.20	43.66
20	47.11	7.68	10.84	59.82
40	47.18	6.42	9.00	66.64
90	47.44	5.70	7.21	73.27
120	47.98	4.18	6.40	76.27
140	49.87	3.72	5.80	78.80
8 % NaOH				
5	38.62	13.00	19.44	27.97
10	39.48	11.39	14.26	47.14
20	40.78	10.88	10.58	60.78
40	43.70	8.67	8.89	67.04
90	44.23	7.12	6.79	74.83
120	44.64	5.98	5.20	82.72
140	50.87	4.66	4.16	84.58
10 % NaOH				
5	44.38	7.18	20.22	25.05
10	45.49	6.40	15.89	41.10
20	47.40	5.88	12.98	51.89
40	48.56	5.11	12.00	55.52
90	49.28	4.86	9.10	66.27
120	50.59	4.32	6.98	74.12
140	51.40	3.90	6.77	74.98

*Treatment time