Evaluation of Different Pretreatment Methods For Hydrolysis of Brewers Spent Grain (BSG)

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Abstract-*Brewers' spent grain (BSG), the main low-value solid residue, is the major by-product of the brewing industry, constituting around 85 %w/w of the total by-products generated. Therefore, BSG apart from being renewable, cheap, and largely available resource throughout the year, has huge potential to add value to the brewing industry. Global pressure to reduce green house gas emission (GHG) and Environmental concerns demanding adoption of green environmental technologies, have seen growing efforts directed towards finding alternative uses of BSG, apart from its current use as an animal feed. In this study, different pretreatment methods were evaluated for hydrolysis of Indian Brewer's Spent Grain (BSG) to obtain the cellulosic and hemicellulosic sugars. Varied range of pretreatment temperatures and retention times were investigated in this work to study the effect of these operating conditions on hydrolysis of BSG. Hemi-cellulose, cellulose, proteins and lignin are the major components of BSG. Arabinose, glucose and xylose were monitored in the reaction products during the hydrothermal treatment. The utilization of simple hydrothermal treatment at a lower temperature (140 ^{0}C) and a reaction time (30 min) resulted in lesser by products formation and lower sugar loss. From the composition of processed BSG, it was calculated that 60 -70 %w/w of the initial complex cellulose and hemicellulose sugars were solubilized in hydrothermal treatment using water as solvent. The study summarizes the observations of different pretreatment studies on the yields and compositions of cellulosic, hemicellulosic and lignin streams which would serve a valuable raw material feed for production of biorefinery chemicals.*

Keywords-Brewer's Spent Grain, Organosolv, Hydrolysis, Acid Hydrolysis

I. INTRODUCTION

Lignocellulose, with an annual worldwide production of 1 × 1010 MT, is the most abundant organic material on earth, accounting for approximately 50%w/w of biomass (1, 2). Any lignocellulosic material (wood, straw, agricultural and industrial materials) has the potential to be a valuable feedstock for various chemicals and biochemical products. However research technologies for realizing this value is still in its infancy albeit evolving with each passing day.

As a result, current use of lignocellulosic feedstock is largely restricted to textiles and paper manufacturing (3), animal feed, or is incinerated for power generation. This feedstock mass has been estimated between 180 million (4), to 1.4 billion tons (5). Nowadays, there is a great political and social pressure to reduce the pollution arising from industrial activities. Almost all developed and underdeveloped countries are adapting processes for maximum residue recycle, reuse or value recovery. As a result, most large companies no longer consider residues as waste, but as a raw material for other processes. The brewing industry generates relatively large amounts of by-products and wastes; spent grain, spent hops and yeast being the most common. However, as most of these are agricultural products, they can be readily recycled and reused. Thus, compared to other industries, the brewing industry tends to be more environmentally friendly (6). Spent grain is the most abundant brewing by-product, corresponding to around 85%w/w of total by-products generated (7). Brewers spent grain (BSG) is available at low or no cost throughout the year, and is produced in large quantities by all large and small Breweries. Recently, attempts have been made to use BSG as a media for cultivation of mushrooms and bacteria viz. actinobacteria, Use of BSG as a source of value-added products, such as, ferulic and p-coumaric acids, xylose, arabinose, is also investigated. Literature also cites examples for use of these streams as raw material for xylitol and arabitol production (8).

Brewery spent grain (BSG), as the name suggests, is a major by product of Brewery industry, contributing more than 85%w/w of the total by produced by the industry. It contains approximately 20%w/w protein and 70%w/w fiber and so can be considered as a potential lignocellulosic resource. . Literature cites some work carried out fractionation of BSG to yield fermentable sugars, however the texture and composition of BSG do pose certain challenges in its effective and commercially feasible fractionation.

Pretreatment of lignocellulosic feedstock helps to breakdown the recalcitrance of the biomass enabling enhanced

hydrolysis of carbohydrates to respective monomeric sugars. (9). In this study we have evaluated a three important pretreatment methods viz. organosolv, direct steam explosion, and the most common acid hydrolysis method for their efficacy in fractionation of BSG. Two important aspects decide the suitability of pretreatment for any feedstock amenable to biological process. These are the availability of free sugars and formation of biological process inhibitors. We have used these two aspects to discuss the comparative performance of these three pretreatments.

II. MATERIALS AND METHODS

Feedstock

BSG was obtained from a local brewery (Bombay Breweries, Taloja Mumbai, India). The BSG obtained was having a moisture content of about 75 %w/w. It was dried at 50^0C to moisture content less than 5 wt %w/w. The feedstock material was then stored in clean dry bags until required for processing or analysis. Solvents of purity 99.5%w/w was purchased from M/s Hayman and M/s Sigma Chemicals ; Sulfuric acid (95-98%w/w purity) was also purchased from M/s Fisher Scientific.

Experimental:

Analytical Methods:

a) Feedstock Analysis: The particle size of reduced to below 1mm using a hammer mill and was subjected to analysis as per standard NREL procedures (11, 12).

The moisture was determined by oven drying at 1050C to constant weight. The mineral components were determined as ash, after incineration at 550ºC. The extractives were determined by ethanol extraction using Soxhlet apparatus [13]. Cellulose, hemicelluloses and lignin were determined by acid hydrolysis of the extractive-free material, followed by chromatographic quantification of the sugars and by products in the hydrolysate stream using HPLC system (Agilent, 1200 , 1260 USA), equipped with UV-VIS and RI detectors. Chromatographic separation was achieved on a Pb-based column (Bio- Rad, Richmond, CA, USA) at 85ºC, using 0.005 M H2SO4 , at a flow rate of 0.6 mL/minute. The acid insoluble fraction was by gravimetrically estimated and reported as acid-insoluble lignin [14]. .

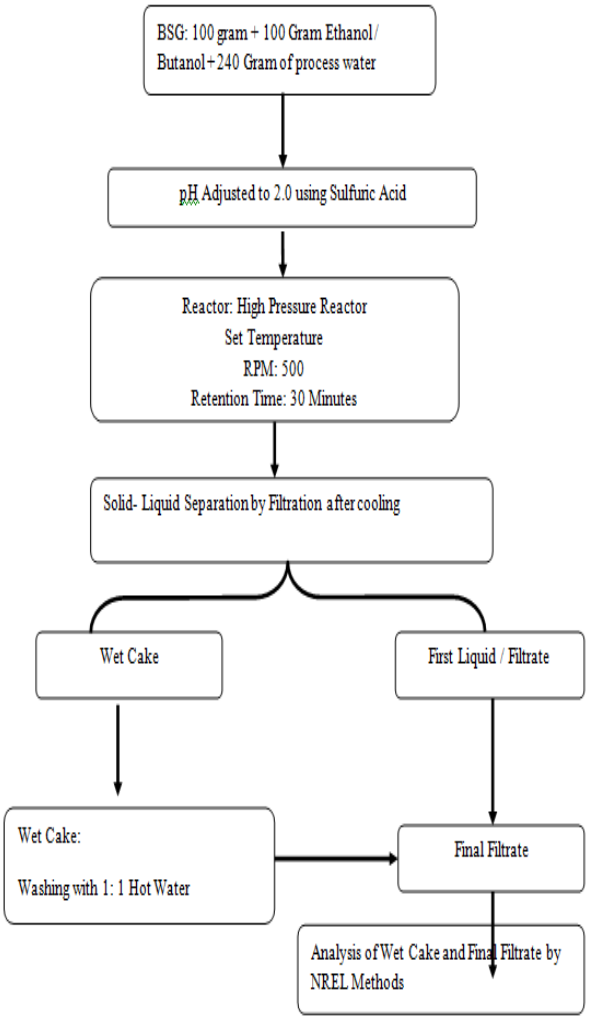
Pretreatment

As mentioned earlier three different methods were evaluated for pretreatment of BSG. Post pretreatment the slurry was subjected to solid –liquid separation. The liquid fraction representing the hydrolysate was analyzed for sugars and by products, whereas the solid cake was analyzed in a manner similar to feedstock analysis. First method evaluated the Organosolv method, wherein hydrolysis of BSG was carried out in presence of an organic solvent, at pH 2 at three different temperatures viz. 120°C, 140°C, 160°C and 180°C for a fixed retention time of 30 minutes duration. Two such sets were carried out using ethanol and n-butanol as organic solvents.

The second method used was steam explosion in absence of any catalyst or solvent, wherein BSG was held at high temperature and pressure for a fixed period of 30 minutes and then flashed at 120 °C from the bottom valve of High Pressure reactor to avoid over dilution of slurry.

Whereas the third approach was the hydrolysis of spent grain at lower pH (pH 1.5) using water as a solvent at four different temperatures 120°C, 140°C, 160° C and 180°C for a fixed period of 30 minutes.

Thus in a typical Organosolv pretreatment batch set-up, 100 g Spent grain was mixed with 80 g organic solvent 320 ml process water was further added to achieve 20 %w/w w/w solids in final slurry. The slurry pH was adjusted to 2.0 using sulphuric acid. The slurry was transferred to a high pressure stirred autoclave. The slurry was cooked at the desired temperature with 150 rpm agitation for a period of 30 minutes. After 30 minutes the autoclave was cooled and the contents were transferred to a Solid-Liquid separator. After solid liquid separation the respective fractions were analyzed for sugars and by products. The detailed Process Flow diagram is depicted in Figure - 1



Figures 1. Organosolv pretreatment

In the second approach involving steam explosion, 500 gram of spent grain was mixed with 500 gram of water in a high pressure reactor. The temperature was raised to 180°C using live steam of 7.5 Kg Bar pressure. Temperature rise from 32°C to 180°C was achieved within 3 minutes. The reaction mixture was held for 30 minutes at 180°C temperature and subsequently flashed at 120°C from the bottom valve of reactor to avoid over dilution of slurry. Solid –liquid separation was carried out using a basket centrifuge. The solid cake obtained was washed with hot water to ensure complete removal of hydrolysate phase. The resultant cake, the liquid phase and the washings were independently analyzed according to NREL standard analytical procedures. Four such experiments were carried out at four different temperature viz. 180°C, 160°C, 140°C and 120°C. The experiment carried out at 120oC was not flashed. Detailed process flow is depicted in Figure 2.

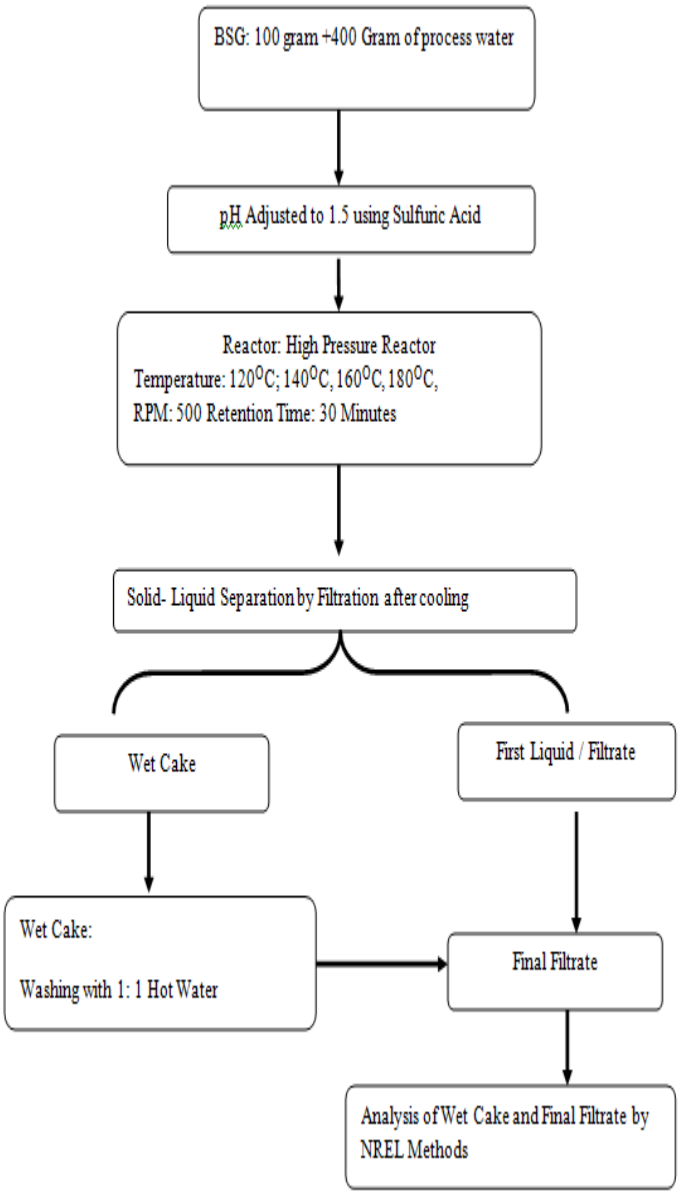


Figure – II Steam Explosion Pretreatment

For acid hydrolysis, the spent grain was mixed with water to achieve a slurry containing 20%w/w solids.The slurry pH was adjusted to 1.5 and then it was transferred to a high pressure autoclave. It was cooked at the desired temperature for 30 minutes, after which the solid and liquid streams were separated and independently analyzed as described above. Detailed process flow is depicted in Figure 3.

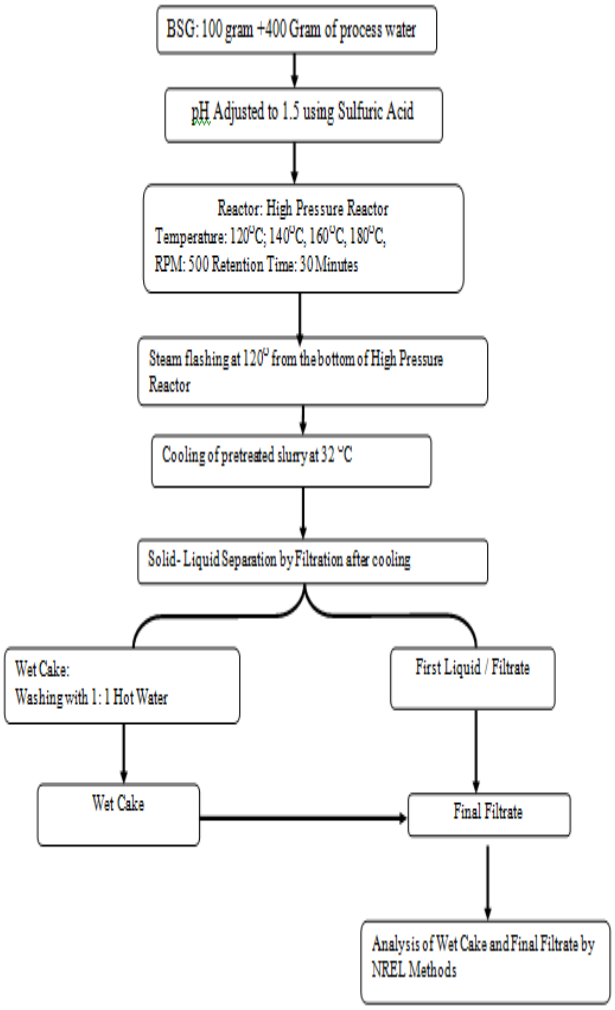


Figure- III – Low pH Pretreatment

III. RESULTS AND DISCUSSION

Compositional Analysis:

Table 1. Composition Analysis of BSG

Sr. No	Parameter	Unit	Test Value
01	Total Solids	%w/w w/w	98.50
02	Moisture	%w/w w/w	1.50
03	Proteins	%w/w w/w	28.85
05	Ash	%w/w w/w	4.13
06	Extractives (Water)	%w/w w/w	8.78
07	Extractives (95 %w/w Ethanol)	%w/w w/w	9.45
08	Cellulose	%w/w w/w	11.37
09	Hemi-cellulose	%w/w w/w	18.68
10	Acetyl	%w/w w/w	0.62
11	Acid Insoluble Lignin	%w/w w/w	14.16
12	Acid Insoluble Lignin	%w/w w/w	4.81

Table 1 depicts typical compositional parameters of BSG. This compositional analysis data is comparable to literature values (13) Since starch forms the major component of hexose sugars in cereals, If we compare BSG with other commonly explored feedstock's for BSG contains comparatively lower amount of cellulosic sugars which are non fermentable. As the starchy component of C6 sugars is completely utilized, a proportionate increase in observed in the concentration of proteins and the hemicellulose. Presence of higher amount of hemicellulose than the cellulose content makes BSG a unique potential feedstock for second generation biofuels and biochemicals. It is well reported in literature (19) that hydrolysis of hemicelluloses is much easier than cellulose, thus there is plenty scope to design processes for selectively converting hemicellose to value added products leaving behind cellulose along with lignin for boiler fuel applications. Lower amount of lignin is also beneficial as it will generate less by products during pretreatment.

IV. PRETREATMENT STUDIES

Results of Organosolv pretreatment are depicted in Table- 2. As expected using ethanol as solvent it is observed that there is marginal increase in retention of sugars in the cake with decrease in temperature from 180^{O}C to 120^{O}C. This is complemented by observations related to sugars recoveries in the liquid fraction where in recoveries of glucose was as high as 14.6 %w/w at 180^{O}C as compare to 8.47 %w/w at 120^{O}C. However the results of sugar recovery depict a particular pattern where in recovery of sugars increase with increase in temperature from 120^{O}C to 160^{O}C. At 160 ^{O}C recovery of sugars in liquid fraction was as high as 22.47 %w/w however further increase in temperature led to rapid drop in glucose recovery from 22.47 %w/w to 14.63 %w/w . This can be attributed to formation of by products. It is observed that HMF which is formed by degradation of glucose increases in concentration from 0.01 %w/w w/w to 0.5 %w/w w/w as temperature is raised from 160OC to 180OC.

Similar observations are also observed in the case of xylose although there is marginal decrease in the xylose retention in the cake with rise in temperature. This decrease is quite remarkable in the retention of arabinose. Its retention was as high as 12.7 %w/w at 120^{O}C and was low as 0.6 %w/w at 180^{O}C. Composition of the liquid fractions show good recoveries of xylose at 140^{O}C where as the recoveries of arabinose are maximum at 120^{O}C Degradation xylose and arabinose is known to form by products such as furfural and acetic acid therefore as expected the concentration these products in liquid fraction significantly increase as the pretreatment temperature was increased from 120°C to 180°C. There has been marginal change in the retention of xylose in the cake so it can be safely assumed that most of the by products are formed due to degradation of arabinose. By product profile of liquid fractions at 160°C and 180°C also shows formation of formic acid which 0.04 %w/w at 160°C and 0.08 %w/w at 180°C. It is well reported in literature that formic acid is product of glucose dehydration where in glucose via HMF gets converted to Levulinic and formic acid.

We have not been able to analyze levulinic acid in our streams.

Change in solvent from ethanol to butanol shows improvement in sugar retention in the cake as butanol being a solvent with high boiling point remains in the liquid phase and being non polar as compared to ethanol helps in proper control of pH . The non polar nature of butanol depresses the acidity and probably would work with more acid quantities. A drastic fall in recovery of glucose is also observed as the temperature is increased from 160°C to 180°C ; at 160°C recovery of glucose is 21.6 %w/w which comes down to 13.73 %w/w at 180°C. As explained above this can be attributed to degradation of sugars which is observed in the byproduct profile of respective streams ; concentration of HMF is 0.05 %w/w at 160°C where as it is as high as 0.19 %w/w at 180°C there is also small amount of formic acid formed with respect to pentose sugars mainly xylose and arabinose. It is observed that recovery of xylose is 39.9 %w/w at 120°C and increases to maximum of 77.6 %w/w at 140°C; further increase in temperature leads to drop in recovery to 63.22 %w/w at 160°C which further drastically drops to nearly 6.28 %w/w at 180°C. Thus it can be assumed that in this system of study rate of xylose degradation into by products occurs at a temperature between 140°C and 160°C and the rate of degradation is exponentially proportional to rise in temperature. This statement is complimented by observing byproduct profile where is the concentration of furfural which is 0.13 %w/w at 140°C increase to 0.3%w/w at 160°C and further increase to 0.42%w/w at 180°C. Xylose degradation is also reflected by the increase in acetic acid concentration from 0.03 %w/w at 140°C to 0.22 %w/w at 180°C. This observation compliments the fall in xylose recovery. In case of arabinose, its recovery increases from 36 %w/w to 48.06 %w/w as temperature is increased from 120°C to 160°C. Further increase in temperature hampers recovery of arabinose and its concentration in the liquid fraction drops to 9.4%w/w at 160°C and further to 3.6%w/w at 180°C.

Table 2. Pretreatment of BSG using Organosolv process

	Solvent	Temperature	Sugar Retention in Cake %w/w			Recovery of Sugars in Liquid Fraction %w/w			By products profile of the liquid fraction %w/w			
			Glucose	Xylose	Arabinose	Glucose	Xylose	Arabinose	Formic acid	Acetic acid	HMF	Furfural
1	Ethanol	180	45.87	21.47	0.6	14.63	48.27	8.26	0.08	0.15	0.05	0.13
2	Ethanol	160	46.5	21.92	1.7	22.47	57.89	11.0	0.04	0.07	0.01	0.08
3	Ethanol	140	47.94	21.57	7.36	10.40	63.30	12.2	0.00	0.02	0.00	0.01
4	Ethanol	120	49.64	25.10	12.76	(8.47	40.48	21.16	0.00	0.01	0.00	0.00
5	n- Butanol	180	47.08	24.20	0.2	13.73	6.28	3.6	0.02	0.22	0.19	0.42
6	n- Butanol	160	49.22	23.03	1.76	21.61	63.22	9.4	0.00	0.06	0.05	0.30
7	n- Butanol	140	51.90	23.62	4.66	8.53	77.63	48.06	0.00	0.03	0.02	0.13
8	n- Butanol	120	53.67	25.90	16.33	6.85	39.92	36.0	0.02	0.00	0.00	0.00

The results of steam explosion pretreatment are summarised in Table -3. It can be observed that glucose retention in the cake is much less as compared to the organosolv pretreatment at all four temperature studied where in 38 %w/w of glucose retained in the cake; this could be due to low pH resulting due to self generated acids and the absence of organic solvent in the liquid phase which cause more hydrolysis of the carbohydrates present. Removal of xylose from BSG increases as the temperature is increased from 120°C to 180°C. 50 %w/w xylose is retained in the cake at 120°C where as removal efficiency increases to more than 90 %w/w at 180°C. A similar observation can be made with respect to arabinose. 88%w/w Arabinose is removed from the cake at 120°C where as at 180°C more than 98%w/w of arabinose is removed from the cake; however as explained earlier rise in temperature also leads to degradation of sugars leading to formation of undesired by products. As compared to organosolv pretreatment due to absence of organic phase and higher expression of acidity, degradation of sugars in the liquid phase in this system is observed at much lower temperature. The recoveries of glucose, xylose and arabinose which are 19.5 %w/w, 21.18 %w/w and 11.45 %w/w respectively at 120°C; increase to 24.95 %w/w, 41.78%w/w and 15.66%w/w respectively at 140°C there after they reduce with increase in temperature to 20.72%w/w, 22.47%w/w and 8.13 %w/w respectively at 180°C. Degradation of sugars leads

to by product formation as explained above and this trend can be very well observed in the by product profile depicted in Table-3. Concentrations of acetic acid and furfural resulting from degradation of xylose increased in the liquid stream gradually from 0.03 %w/w and 0.02 %w/w respectively at 120°C to 0.16 %w/w and 0.13 %w/w respectively at 180°C. A similar increase is observed in concentration of HMF and formic acid formed from glucose decomposition with rise in temperature. Pretreatment at 140°C does not show formation of formic acid and HMF. However as the temperature was increased to 160°C and further to 180°C concentration of formic acid increased to 0.04 %w/w and 0.08 %w/w respectively where as acetic acid concentration increased from 0.07 %w/w and further to 0.16 %w/w respectively.

Table 3. Pretreatment of BSG using steam explosion

	Temp.	Sugar Retention in Cake %w/w			Recovery of Sugars in Liquid Fraction %w/w			By products profile of the liquid fraction %w/w			
		Glucose	Xylose	Arabinose	Glucose	Xylose	Arabinose	Formic acid	Acetic acid	HMF	Furfural
1	180	29.74	10.05	1.66	8.16	17.3	3.27	0.08	0.16	0.05	0.13
2	160	32.63	28.64	7.36	20.72	22.47	8.13	0.04	0.07	0.01	0.08
3	140	34.19	35.12	10.76	24.95	41.78	15.66	0.01	0.02	0	0.01
4	120	38.64	49.95	12.13	19.5	28.18	11.45	0.01	0.03	0	0.02

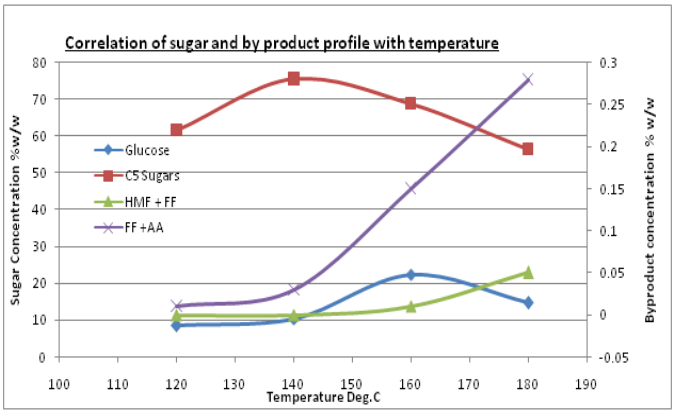
The third set of pre treatment involves indirect heating of BSG at pH 1.5.As compared to steam explosion there was more glucose retained in the cake. The probable reason could be lesser penetration of pretreatment liquid in the BSG biomass resulting in inefficient mass transfer; around 45 %w/w glucose is retained in cake which is higher as compared to retention of glucose in steam explosion which is around 38 %w/w. However xylose and arabinose which are easily accessible to acid pretreatment are efficiently removed. Their efficiencies are almost comparable to those observed in steam explosion process. The correlation of xylose and arabinose removal efficiency with respect to temperature is similar to that observed in earlier organosolv and steam explosion pretreatments. The xylose retained in cake was 32.9 %w/w at 120°C where as pretreatment at 180°C resulted in more than 90%w/w xylose removal. Formation of by products is observed in this system too due to increase in temperature. This pretreatment has a low operating pH thus enables recovery of xylose and arabinose at 120°C which gradually reduces to formation of by products. The results of this pretreatment are summarised in Table – 4

Table 4. Pretreatment of II - BSG using sulfuric acid at pH 1.5

	Temp.	Sugar Retention in Cake %w/w			Recovery of Sugars in Liquid Fraction %w/w			By products profile of the liquid fraction %w/w			
		Glucose	Xylose	Arabinose	Glucose	Xylose	Arabinose	Formic acid	Acetic acid	HMF	Furfural
1	180	45.96	9.14	6.53	5.77	26.35	0.00	0.00	0.12	0.17	0.59
2	160	45.17	19.14	7.28	7.09	31.36	0.00	0.00	0.08	0.10	0.29
3	140	44.33	32.90	8.63	11.83	41.73	16.26	0.00	0.05	0.01	0.16
4	120	46.66	31.91	9.60	12.64	64.4	18.97	0.00	0.02	0.00	0.00

The correlation of sugar and byproducts with respective temperature has been studied which shows that increase of pretreatment temperature results in byproducts formation as shown in graph – 1

Graph – 1



C5 Sugars = Xylose, Arabinose, HMF = Hydroxy Methyl Furfural, FF = Furfural , AA = Acetic Acid

V. CONCLUSION

Choice of pretreatment is governed by the type of process that needs to be implemented for further conversion of sugars to value added products. High temperature pretreatment results in low recovery of sugars which is attributed to formation of humans due to acidity and high temperature. Referring to all three pretreatments the recovery of sugars is maximum at 120°C and 140°C; temperature higher that 140°C results into formation of humins. Comparing glucose with pentose sugars namely xylose and arabinose it is well known that glucose is more prone to humins formation hence it is observed that recovery of glucose is always less where as recovery of xylose is almost quantitative at lower temperature of 120°C to 140°C . Organosolv treatment results in better recovery of glucose as compare to steam explosion and acid hydrolysis The presence of organic solvent makes the pretreatment media less polar there by controlling the reaction which leads to formation of humins.

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