Optimization of Pretreatment of Wheat Straw Using Alkali and Biphasic Acid Hydrolysis

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ABSTRACT

Pretreatment is an important step in the conversion of biomass to biofuels. Alkali pretreatment increases cellulose digestibility and it is the most effective method for lignin solubilization, exhibiting minor cellulose and hemicellulose solubilization compared to acid or hydrothermal processes. The present study describes the potential of pretreatment of wheat straw to degrade lignin and facilitate conversion of polymers to single monomers using alkali treatment followed by biphasic acid hydrolysis. Two alkalis (NaOH and KOH) at various concentrations (0.25, 0.5, 0.75 and 1 M) and at two incubation periods (6 and 12 h at room temperature) were used to delignify wheat straw. 1 M NaOH incubated for 6 h significantly degraded lignin (77%) with minimum sugar loss (1.6%). Further, delignified wheat straw was subjected to two different biphasic acid hydrolyses; firstly, biphasic acid hydrolysis was performed with 2% sulphuric acid at 121°C for 1 h followed by 4% sulphuric acid at 121°C for 1 h. Secondly, biphasic acid hydrolysis was performed with 3% sulphuric acid followed by 4% sulphuric acid under the same conditions. The conversion of holocellulose (cellulose + hemicellulose) during the above two different biphasic acid hydrolyses was 52 ± 2 and $41 \pm 2\%$, respectively.

INTRODUCTION

Worldwide attention has recently turned to bioethanol production as a strategy to combat global warming and to improve global energy security. However, feedstocks of current bioethanol production methods are currently derived from edible parts of food crops such as sugarcane, rice straw, wheat straw and corn (Sakai *et al.* 2007). Lignocellulosic materials are abundant and cheap feed stock, but the processing techniques required for ethanol production are expensive. The cost of ethanol produced from lingocellulosic materials with currently available technology under the present economic conditions is not competitive with the cost of gasoline. Comprehensive process development and optimization are still required to make the process economically viable.

Wheat straw is annually generated in abundance worldwide (529 million tons/year) (Kim and Dale 2004). This huge amount of residues may constitute a promising raw material that could potentially be transformed into a more edible feed for ruminants or alternatively it could also be used for the production of ethanol (Rodrigues *et al.* 2008).

The cell wall of wheat straw consists mostly of cellulose fibres. Cellulose is a linear crystalline polymer of (1-4)-β-D-glucose. Hemicellulose is an amorphous and partly crystalline polymer, which mostly consists of (1-4)-β-xylose. Lignin, a phenolic polymer, binds the fibres together. Hemicellulose and lignin protects the cellulose and this leads to pretreatment of wheat straw before the hydrolysis. It binds to side chains of xylans of cell wall and it hinders the microbial digestion of carbohydrates (Antongiovanni *et al.* 1991). Depending on the composition of guaiacyl (G), syringyl (S) and p-hydroxyphenylpropane (H) units, wheat straw lignin has been justified as GSH-lignins (Gramineae lignins from grasses), which are known to be different from those of softwood (G-lignins), hardwood (GS-lignins) and compression wood (GH-lignins) lignins. Moreover, phenolic acids, mainly p-coumaric and ferulic acids have been suggested to form cross-links between lignins and hemicelluloses, thus participating in the reticulation of the wheat straw cell walls (Billa and Monties 1995).

In the present study wheat straw was used as the lignocellulosic substrate since it represents an abundant renewable energy source for bioconversion processes as well as raw material for the production of ethanol (Fang *et al.* 2002). Pretreatment of the biomass is required to hydrolyze the hemicellulose and make the cellulose more accessible. An efficient pretreatment system is crucial to the enzymatic or acid hydrolysis and thus the fermentation process, which are the essential steps in the cellulose conversion process to produce bioethanol (Mosier *et al.* 2005). The present study consists of two separate parts, alkali pretreatment and biphasic acid hydrolysis of wheat straw. Alkali extraction causes solubilization of lignin and modifications in the crystalline state of the cellulose (Carvalheiro *et al.* 2008). These effects can lower or counteract the positive effects of lignin removal (Gregg and Saddler 1996). According to Taherzadeh *et al.* (2008), NaOH causes swelling, increasing the internal surface of cellulose and decreasing the degree of polymerization and crystallinity, which provokes lignin structure disruption. Biphasic hydrolysis studies were conducted to separately hydrolyze hemicellulose and cellulose fractions present in the wheat straw (Lee *et al.* 2009). Dilute acid hydrolysis was done in the first step as it can achieve high reaction rates and significantly improve cellulose hydrolysis (Esteghlalian *et al.* 1997). In the second step, concentration of acid was increased to get possibly the remaining sugars. Moderate temperature (121°C) was applied in both the steps of acid hydrolysis since higher temperatures in dilute acid hydrolysis cause degradation of monosaccharides and formation of inhibitor compounds like furans, weak carboxylic acids and phenolics which ultimately inhibit the fermentation process (Olsson and Hahn-Hagerdal 1996; Larsson *et al.* 1999; Klinke *et al.* 2004).

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MATERIALS AND METHODS

Raw material

Wheat straw was collected from Aligarh, Uttar Pradesh, India. The straw was dried and processed in a laboratory pulverizer to attain a particle size between 1 and 3 mm followed by sieving. Then it was washed with running tap water to make it clear and dust free and then oven dried at $60 \pm 0.5°C$.

Analysis of chemical composition of wheat straw

The cellulose, lignin and hemicellulose fractions of pulverized wheat straw were determined according to the Van Soest *et al.* (1991) method. Wheat straw was grinded in a pulverizer through a 1 mm screen and then dried at 50°C for 24 h. The straw was then analyzed in triplicate for neutral detergent fibre (NDF) and acid detergent lignin (ADL) using the procedure of Van Soest *et al.* (1991). Hemicellulose was calculated as a difference between NDF and ADL. Cellulose, lignin and ash were determined according to the above method.

Alkali pre-treatment

The alkali pretreatment method was carried out by taking 50 g chopped and dried wheat straw at 10% (w/v) in 500 ml of alkali (KOH and NaOH) of various concentrations (0.25, 0.5, 0.75 and 1 M) at room temperature (30 ± 2°C) for two different incubation periods (6 and 12 h). The delignified filtrate was pressed through cheese cloth and the amount of reducing sugars and phenolics in the filtrate were measured by using DNS (Miller 1959) and Folin Ciocalteau's method (Singleton *et al.* 1965), respectively. The residual substrates were washed and neutralized with water and then oven dried at 50°C for further acid hydrolysis.

Biphasic acid hydrolysis

The KOH and NaOH pretreated wheat straw was subjected to two different biphasic acid hydrolyses at 10% (w/v) substrate. First biphasic acid hydrolysis was done with 2% sulphuric acid at 121°C for 1 h followed by 4% sulphuric acid at 121°C for 1 h. The second biphasic acid hydrolysis was performed with 3% sulphuric acid followed by 4% sulphuric acid using the same conditions. The hydrolysate obtained was analyzed for the amount of reducing sugars and phenolics present in it.

Analytical methods

Total reducing sugars present in delignified wheat straw filtrate and acid hydrolysate were estimated by the dinitrosalicylic acid method (Miller 1959). The phenolics were estimated by Folin Ciocalteau's method (Singleton *et al.* 1965).

Statistical analysis

To assess whether there was any significant difference between the concentration of alkali and percentage of delignification and between concentration of acid and concentration of sugars obtained, a correlation test and two paired sample *t*-tests were performed using SPSS (software for windows release, 17.0, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Analysis of chemical composition of wheat straw

The wheat straw samples were analysed for chemical composition in triplicates (Van Soest *et al.* 1991). The average percentages of moisture, lignin, cellulose, and hemicellulose were found to be 8.3, 19.5, 33.5 and 25.5%, respectively. The percentages of holocellulose and ash were 59 and 10.8%, respectively.

Alkali pre-treatment

Alkali pretreatment showed effective lignin solubilization exhibiting minor cellulose and hemicellulose solubilization. Among the two alkali treatments (NaOH and KOH), treatment with NaOH showed better delignification. In all the pretreatment experiments with increasing concentrations of alkali, the recovery of lignin increased slightly. Comparing the treatment of wheat straw with NaOH and KOH at various concentrations (0.25, 0.5, 0.75 and 1 M), a higher lignin solubilization performance was observed when the wheat straw was treated with 0.75 M NaOH and 1 M NaOH at 6 h (76% ,77%) (**Fig. 1**) and 12 h (77%, 79%) (**Fig. 2**) of incubation. Prolonging pretreatment time (up to 12 h) at the same concentration led to a little increase in lignin degradation. Cheng (1993) reported 60-70% of lignin removal from wheat straw by using 1.5% NaOH at 100°C for 1 h. Though the sugar loss is slightly higher, it is < 5%. Free phenolic monomers in each of the alkaline treatment hydrolyzates were isolated and analyzed. Though the percentage of delignification was slightly higher at 12 h of incubation, the difference is insignificant.

NaOH loading had a very significant influence on the degree of delignification. Lignin degradation dramatically increased with NaOH loading. These findings imply that higher NaOH loading can remove more lignin and also dissolve fewer carbohydrates. According to these experimental results, 1M NaOH loading at room temperature for 6 h was optimum.

Biphasic acid hydrolysis

The best alkali pretreated residual substrate was subjected to two different biphasic acid hydrolyses. Concentrated acid such as H_2SO_4 was used to treat delignified wheat straw as
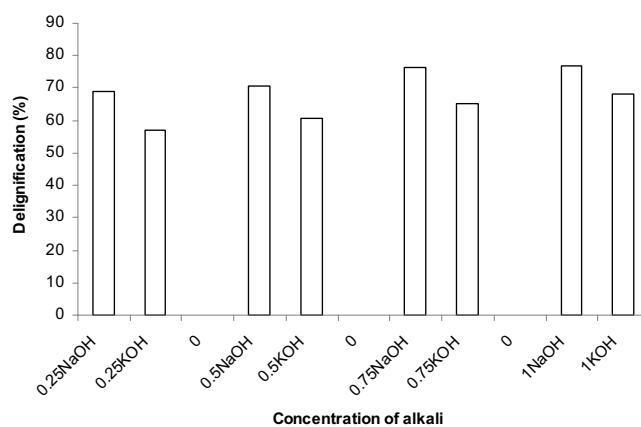

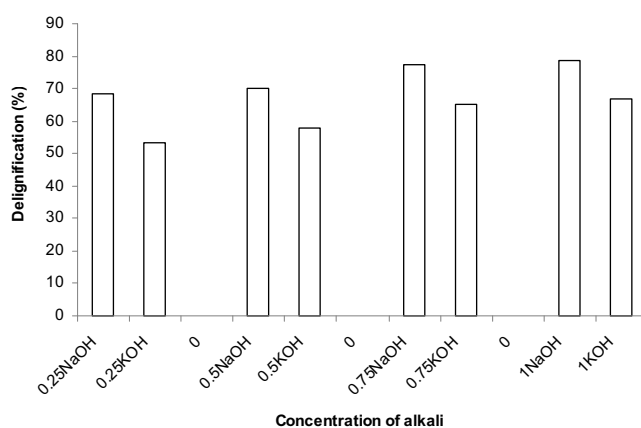**Fig. 1 Delignification of wheat straw using different concentrations of NaOH and KOH at 6 h.**

Fig. 2 Delignification of wheat straw using different concentrations of NaOH and KOH at 12 h.
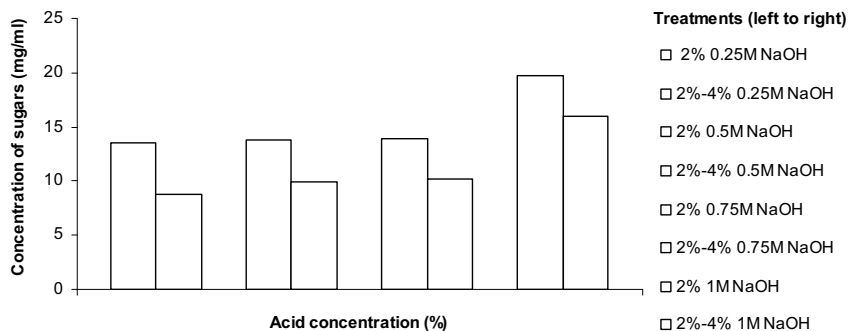
Treatments (left to right)

☐ 2% 0.25M NaOH
☐ 2%-4% 0.25M NaOH
☐ 2% 0.5M NaOH
☐ 2%-4% 0.5M NaOH
☐ 2% 0.75M NaOH
☐ 2%-4% 0.75M NaOH
☐ 2% 1M NaOH
☐ 2%-4% 1M NaOH

Fig. 3 Sugars released during biphasic acid hydrolysis (2% followed by 4%).

Treatments (left to right)

☐ 3% 0.25M NaOH
☐ 3%-4% 0.25M NaOH
☐ 3% 0.5M NaOH
☐ 3%-4% 0.5M NaOH
☐ 3% 0.75M NaOH
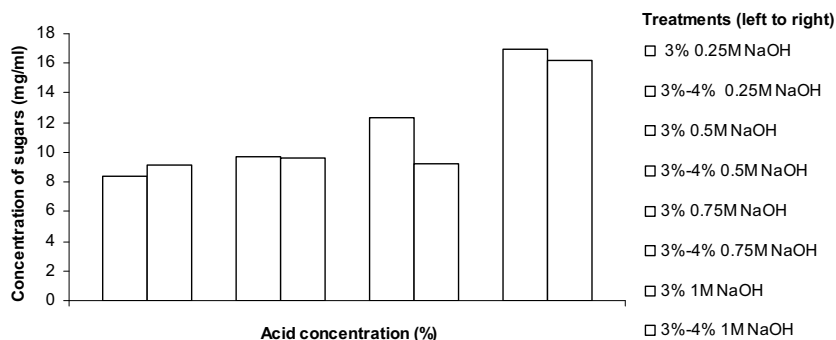☐ 3%-4% 0.75M NaOH
☐ 3% 1M NaOH
☐ 3%-4% 1M NaOH

Fig. 4 Sugars released during biphasic acid hydrolysis (3% followed by 4%).

Table 1 Sugars present in biphasic acid hydrolysate (2% followed by 4%).

Alkali pretreated substrate	2%+4%AH Sugar concentration (mg/ml)	2%+4% AH Sachharification
0.25 M NaOH	23.90	29
0.5 M NaOH	23.60	29
0.75 M NaOH	22.40	27
1 M NaOH	35.60	52

Table 2 Sugars present in biphasic acid hydrolysate (3% followed by 4%).

Alkali pretreated substrate	3%+4%AH Sugar concentration (mg/ml)	3%+4% AH Sachharification
0.25 M NaOH	16.80	21
0.5 M NaOH	19.33	24
0.75 M NaOH	12.40	13
1 M NaOH	33.23	41

it is a powerful agent for cellulose hydrolysis. In addition, concentrated acid must be recovered after hydrolysis to make the process economically feasible (von Sivers and Zacchi 1995).

Figs. 3 and **4** show the yields of sugars produced by 2 and 3% sulphuric acid hydrolysis of wheat straw delignified by various concentrations of NaOH (since NaOH showed maximum delignification, NaOH treated wheat straw was used further in acid hydrolysis studies). Dilute sulphuric acid at moderate temperature (60-110°C) effectively removes and recovers most of the hemicellulose as dissolved sugars (Lu *et al.* 2008). The present results also indicate that dilute sulphuric acid treatment (2 and 3%, v/v) effectively released a maximum amount of sugars from wheat straw. The reducing sugar yields of delignified wheat straw with 1 M NaOH in the first step *i.e.*, 2 and 3% of the two biphasic hydrolysis methods were 19.6 gL^{-1} (24.1%) and 17 gL^{-1} (20.9%), respectively. Khokhar *et al.* (2010) reported 17.71 gL^{-1} yield after 2% sulphuric acid hydrolysis at 121°C for 1 h. The sugar yield in the second step *i.e.*, 4% sulphuric acid hydrolysis in both biphasic acid hydrolysis methods were 15.93 gL^{-1} (23.6%) and 16.23 gL^{-1} (22.9%), respectively (**Figs. 3, 4**). Schmidt *et al.* (1998) reported 8.8 gL^{-1} in 4% sulphuric acid hydrolysis at 121°C for 1 h although in a sin-

gle phase. Besides sugars, the hydrolyzate contained different and varying amounts of toxic components like furfural, soluble lignin derivatives which are known to inhibit microorganisms to produce ethanol.

The total yield of sugars in first biphasic acid hydrolysis *i.e.*, 2% acid hydrolysis followed by 4% acid hydrolysis was 35.4 gL^{-1} (52%) (**Table 1**) and the sugar yield in the second biphasic hydrolysis was 27.9 gL^{-1} (41%) (**Table 2**).

Statistical evaluation of delignification and biphasic acid hydrolysis

To assess whether there was any significant correlation between the percentage delignification and concentration of alkali, a correlation test was performed (**Table 3**). As there was a positive correlation (Pearson correlation = 0.582) between the percentage of delignification and concentration of alkali at 95% confidence interval (sig: 0.018, $P < 0.05$), the percentage of delignification increased with an increase in the concentration of alkali. **Table 4** shows the paired *t*-test which was performed to assess differences between the mean values of percentage of delignification by NaOH and KOH at 6 and 12 h. Since there was no significant difference between 6 and 12 h of treatment with both NaOH

Table 3 Statistical evaluation (Pearson's correlation) of concentration of alkali and percentage delignification.

	Mean	Std. Deviation	N		Concentration of alkali	Percentage of delignification
concentration of alkali	0.6250	0.28868	16	Pearson Correlation	1	0.582*
percentage of delignification	67.6031	7.57442	16	Pearson Correlation	0.582	1

* Correlation is significant at the 0.05 level (2-tailed). n = 16

Table 4 Statistical evaluation (paired samples test) of percentage delignification by NaOH and KOH after 6 and 12 h.

		Mean	Mean difference	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
Pair 1	percentage delignification by NaOH after 6 h	73.1232	-.40619	8.85376	2.55586	-6.03160	5.21922	-0.159	11	0.877
	percentage delignification by NaOH after 12 h	73.5294								
Pair 2	percentage delignification by KOH after 6 h	62.5655	1.68186	8.52571	2.46116	-3.73512	7.09884	0.683	11	0.509
	percentage delignification by KOH after 12 h	60.8836								

Table 5 Statistical evaluation (paired samples test) of concentration of sugars by biphasic acid hydrolsis (2% acid followed by 4% and 3 % acid followed by 4%).

	Mean	Mean difference	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
concentration of sugars 2+4	13.2082	3.00518	2.71614	0.55443	1.85825	4.15211	5.420	23	0.000
concentration of sugars 3+4	10.2030								**

** Mean difference is significant at the 0.01 level (2-tailed).

(sig: 0.877, $P > 0.05$) and KOH (sig: 0.509, $P > 0.05$), a 6-h incubation period was selected for further studies.

Another paired *t*-test was performed (**Table 5**) to determine the difference between the mean values of concentration of sugars and the percentage of acid used in biphasic acid hydrolysis using sulphuric acid (2% acid followed by 4 and 3% acid followed by 4% sulphuric acid hydrolysis). Since there was a significant difference between the means of concentration of sugars in 2% acid hydrolysis followed by 4 and 3% followed by 4% at a 99% confidence interval (sig-0.000 $P > 0.01$), 2% followed by 4% acid hydrolysis was found to be the best method for the maximum saccharification of wheat straw.

CONCLUSION

Pretreatment is a necessary element in bioconversion of lignocellulosics to fuels and other chemicals. Although various chemical methods of pretreatment for cellulosic materials have been proposed, their effectiveness varies, depending on the substrate. Hence, a suitable pretreatment must be optimized for each substrate. In the present study optimization and comparison of different variables for pretreatment and acid hydrolysis have been carried out successfully. A maximum of 77% delignification was achieved using 1 M NaOH at room temperature for 6 h. The highest sugar concentration of 35.4 gL^{-1} representing 52% of holocellulose was released by hydrolyzing the substrate at 121°C for 1 h in two phases *i.e.*, 2% followed by 4% sulphuric acid. Instead of harsher conditions, mild reaction temperatures were applied during delignification and acid hydrolysis to avoid the degradation of sugars.

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