

FTIR and SEM analysis of thermo-chemical fractionated sugarcane bagasse

[Termo-kimyasal olarak fraksiyonlara ayrılmış şeker kamışı küspesinin FTIR ve SEM analizi*]

Muhammad Irfan,
Quratulain Syed,
Sajjad Abbas,
Muhammad Gul Sher,
Shahjahan Baig,
Muhammad Nadeem

Food & biotechnology research Center, Pakistan
Council of Scientific & Industrial Research
(PCSIR) Laboratories Complex, Ferozpure, Road
Lahore, Pakistan

Yazışma Adresi
[Correspondence Address]

Quratulain Syed

Principal Scientific Officer,
Biotechnology and Food Research Centre, PCSIR
Labs. Complex, Lahore-54600, Pakistan
Tel: 0092429231834
Fax: 0092429231835
E-mail: quratulainasad@yahoo.com

*Translated by [Çeviri] Özlem Dalmızrak

Registered: 4 November 2010; Accepted: 17 October 2011
[Kayıt Tarihi : 4 Kasım 2010; Kabul Tarihi : 17 Ekim 2011]

ABSTRACT

Objective: The major objective of this study was to increase the delignification of sugarcane bagasse by using suitable concentration of KOH after specific heating time.

Materials and Methods: Pretreatment of sugarcane bagasse was done in 250 ml conical flask containing ten grams of chopped bagasse mixed with various concentrations of KOH in ratio of 1:10 (solid to liquid) for various time of heating at 121°C and 15lb psi. After heating the slurry was filtered, washed and then dried. The dried biomass was analyzed for delignification. Changes in structure was also observed microscopically (SEM) and spectroscopically (FTIR) leading to enhance the enzymatic digestibility of the sugarcane bagasse.

Results: Maximum delignification (70.7%) was achieved with 2.5% KOH at autoclaving time of 45 min. Total sugars (192.32 mg/ml) released with 1.5% KOH at 30 min of autoclaving time and maximum weight loss (57.4%) was observed with 4% KOH at autoclaving time of 15 min.

Conclusion: Alkaline treatment by using KOH of lignocellulosic materials caused swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure. For efficient enzymatic hydrolysis and ethanol production, pretreatment is an important technique which accelerates the enzymatic reaction.

Key Words: Alkaline pretreatment, sugarcane bagasse, saccharification

ÖZET

Amaç: Çalışmada uygun KOH derişimi ve ısıtma süresi kullanılarak şeker kamışı küspesindeki ligninin uzaklaştırılma veriminin artırılması amaçlanmıştır.

Yöntem ve Gereçler: Şeker kamışı küspesinin ön muamelesi 250 ml'lik konik kaplarda gerçekleştirilmiştir. Bu kaplarda, 10 gr kıyılmış şeker kamışı küspesi değişik KOH derişimleri ile 1:10 (katıdan sıvıya) oranında karıştırılmış ve 121°C ve 15lb psi'de değişik sürelerde ısı ile muamele edilmiştir. Isıtma sonrasında örnek filtrelenerek yıkanmış ve kurutulmuştur. Kurutulmuş biyokütle ligninin uzaklaştırılması bakımından analiz edilmiştir. Yapısal değişiklikler mikroskobik (SEM) ve spektroskopik (FTIR) olarak da gözlenmiştir.

Bulgular: En fazla lignin uzaklaştırması (%70.7) %2.5 KOH derişimi ve 45 dakika otoklavlama süresi ile sağlanmıştır. Toplam şeker salınımı (192.32 mg/ml) %1.5 KOH ve 30 dakika otoklavlama ile maksimum ağırlık kaybı ise (%57.4) %4 KOH ve 15 dakika otoklavlama süresi ile elde edilmiştir.

Sonuç: Lignoselulozik materyallere KOH kullanılarak yapılan alkali muamele şişmeye ve buna bağlı olarak iç yüzey alanının artmasına, polimerizasyon derecesinin ve kristal özelliğinin azalmasına ve ayrıca lignin ve karbohidratlar arasındaki yapısal bağlantıların ayrılmasına ve ligninin yapısını kaybetmesine yol açmaktadır. Etkili enzimatik hidroliz ve etanol üretimi için enzimatik reaksiyonu hızlandıran ön muamele önemli bir tekniktir.

Anahtar Kelimeler: Alkali ön muamele, şeker kamışı küspesi, sakkarifikasyon

Introduction

In recent years, there has been an increasing trend towards more efficient utilization of agro-industrial residues, including sugarcane bagasse. Several processes and products have been reported that utilize sugarcane bagasse as a raw material. These include electricity generation, pulp and paper production, and products based on fermentation. Bagasse could also have been used for the production of fuel grade ethanol. However, processes involving bagasse for ethanol production required in substantial quantity. This would affect the supply of fuel for the sugar mills and would necessitate the search for an alternative fuel for them, which has so far largely been unsuccessful [1]. Pretreatment is the first step for conversion of biomass into ethanol. Pretreatment is necessary step for efficient conversion because plant cell walls are made up of very hard coverings like lignin, hemicelluloses and cellulose. Various pretreatment methods are used for treating the biomass but alkaline treatment of sugarcane bagasse digests the lignin matrix and makes cellulose and hemicellulose available for enzyme degradation [1-3]. A thermochemical pretreatment of bagasse involved autoclaving with a binary solvent, composed of water and organic solvent having an upper critical temperature (UCT) on the mutual solubility curve. The pretreatment was termed as UCT-solvent pretreatment and proved to be of significant potential [4]. Alkaline hydrogen peroxide treatment of bagasse was also found effective in improving its digestibility [5,6]. The present study was conducted to optimize the concentration of KOH which was necessary for maximum delignification of sugarcane bagasse to expose cellulose for saccharification process.

Materials and Methods

Lignocellulosic biomass

Sugarcane bagasse was used as a source of lignocellulosic biomass which was provided by Shakar Gunj Sugar mills (Pvt.) Limited, Jhang Road, Faisalabad. The biomass was washed and dried to remove the unwanted particles and then milled into powder form (2mm) with hammer beater mill.

Pretreatment of substrate

Pretreatment of the substrate was performed as described earlier [7]. The chopped sugarcane bagasse samples were soaked in different concentration of KOH ranging from 1-4% solution at the ratio of 1:10 (solid:liquid) for 2 hr at room temperature. After then the samples were autoclaved at 121°C for various time periods (15, 30, 45, 60 min). Then samples were filtered and solid residues were washed up to neutrality.

Chemical analysis

For total sugar determination, 0.5 ml of sample solution was mixed with 0.5 ml of 5% phenol solution and vor-

texed. Concentrated H₂SO₄ (2.5 ml) was quickly added and vortex mixed. The optical density was recorded at 490 nm [8]. Total sugar content was calculated from the standard curve obtained from a series of glucose solutions as standard sugar. Reducing sugars in the filtrate were estimated by adding 0.5 ml of sample solution to 1.5 ml of 3,5 dinitrosalysilic acid. The reaction mixture was then placed in boiling water bath for 10 min. After completion of the reaction, cooled at room temperature and the absorbance was read out at 550 nm spectrophotometrically [9]. The lignin content in treated and untreated samples were measured by considering lignin in the remaining solid residue after hydrolysis with 1.25% H₂SO₄ for two hours and 72% H₂SO₄ hydrolysis for four hours. The residues was filtered and washed with distilled water to remove sulphuric acid and oven dried at 105°C for constant weight. The lignin was expressed by using the formula [10]. Cellulose in the untreated and treated sugarcane bagasse was estimated by weighing 1 g of oven dried samples in round bottom digestion flask, 15 ml of 80% acetic acid and 1.5 ml of concentrated HNO₃ were added to the flask and refluxed for 20 min. After refluxing the material was filtered through Whatmann filter paper #1 and washed with hot water. After washing the digested material was oven dried at 105°C overnight and weighed then incinerated at 550°C for 5 h in muffle furnace and weighed again [11]. The percentage of cellulose on dry matter basis can be calculated using the following formula.

Cellulose (%) Dry matter basis = $\frac{\text{Weight of Digested Material} - \text{Weight of Ash}}{\text{Weight of material on dry basis}}$

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

Scanning electron microscopy of sugarcane bagasse

Samples of untreated and treated sugarcane bagasse were oven dried at 50°C for 1 h and thick layers were supported in the sample holder fixed on a carbon ribbon. This assembly was maintained in a vacuum-desiccator until the analysis. The SEM type S-3700 microscope (Hitachi) was used for observing the bagasse fibers in both treated and untreated samples.

Fourier transform infrared spectroscopy (FTIR)

FTIR was used to check the chemical changes in treated and untreated samples. Mixture of sample and KBr (5% sample : 95% KBr) were passed into a disk for Fourier Transform Infrared Spectroscopy measurement. The spectra were recorded with 32 scans in the frequency range of 4000-400 cm⁻¹ with a resolution of 4 cm⁻¹.

Statistical analysis

All measured values are the averages of three replicates. Values in the figures are means of 3 replicates ± standard deviation.

Results and Discussion

Compositional analysis of bagasse

The compositional analysis of sugarcane bagasse was shown in Table 1. The results indicates that sugarcane bagasse consist of 40% cellulose, 23% lignin, total sugars, reducing sugars, ash and moisture content. Some workers reported that sugarcane bagasse contained cellulose content of 40% and 25% of lignin dry basis [12,13]. Other studies also represent that grasses consist of 25-40% of cellulose and 35-50% of hemicellulose and 10-30% of lignin content [14-17]. Rodríguez-Chong *et al.* [18] also examined the compositional analysis of sugarcane bagasse which comprised of 38.9% cellulose, 20.6% xylan and 23.9% lignin. In another study the cellulose content of 34.3% and ash content of 1.4% was reported in raw sugarcane bagasse [19]. Zandersons *et al.* [20] reported that the oven dried Brazilian sugarcane bagasse contained 3.2% ash and 25.4% klason lignin. These variations in the compositional analysis of sugarcane bagasse might be due to the different varieties which are cultivated in various parts of the world. Hemicellulose, cellulose and lignin are the three main components of biomass which varies from 20–40, 40–60, and 10–25 % respectively for lignocellulosic biomass [21].

The biodegradability of lignocellulosic biomass is limited by several factors like crystallinity of cellulose, available surface area, and lignin content. Cellulose and hemicellulose are the major components of the secondary layers of the cell wall in lignocellulosic fibres [22]. When the bagasse samples were analyzed for cellulose content after every treatment, it was observed that maximum cellulose content (83%) was obtained with 4% KOH for 15 min of autoclaving time as shown in the Figure 1. The isolated cellulose fiber treated with 10% KOH and 10% NaOH yielded 44.7 and 44.2% cellulose (C3, C4), in which 92.5 and 95.5% of the original hemicelluloses were released [23]. They reported that cellulose purity was increased as the strength of KOH, NaOH and temperature was increased. This significant solubility of hemicelluloses was probably due to the presence on outer fiber surface, from where they dissolve easily in the alkaline solution while cellulose fibers are located in the inner parts of the fibres and therefore is not easily dissolved. An important aspect of alkaline pretreatment is that it changes the structure of cellulose fiber, making it denser and thermodynamically stable than the native fiber [24]. Alkaline extraction can also cause solubilization, redistribution and condensation of lignin and modifications in the crystalline state of the cellulose. These effects can lower or counteract the positive effects of lignin removal and cellulose exposure [25].

Figures 2 and 3 represent the sugars released after treatment with various concentrations of KOH at various autoclaving times. Maximum total sugar (192.32 mg/ml and 163.4 mg/ml) was released during treatment with 1.5% and 4% KOH at 30 min of heating time respectively

Table 1. Proximate analysis of the sugarcane bagasse (% w/w of the dry matter)

Content	%
Cellulose	40 ± 1.2
Lignin	23 ± 1.01
Total Sugars	2.94 ± 0.3
Reducing sugars	8.05 ± 0.4
Ash	0.9 ± 0.02
Moisture	7.1 ± 0.5

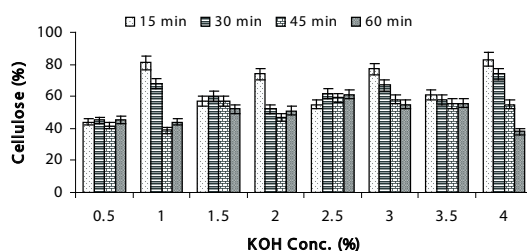


Figure 1. Effect of different conc. of KOH and steaming time on Cellulose content of sugarcane bagasse

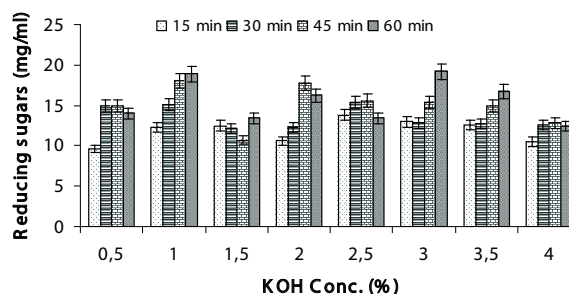


Figure 2. Effect of different concentrations of KOH and steaming time on release of reducing sugars from sugarcane bagasse

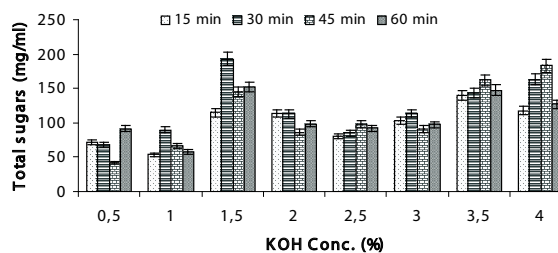


Figure 3. Effect of different concentrations of KOH and steaming time on release of total sugars from sugarcane bagasse

which indicates the maximum degradation of hemicellulose in the biomass. Reducing sugars released were 19.2 mg/ml and 18.9 mg/ml with concentration of 3% and 1% KOH at autoclaving time of 60 min. By increasing the concentration of loading NaOH, the delignification rate will be increased and enzymatic hydrolysis will be better and maximum sugars were released during this

hydrolysis [26]. The production of fermentable sugars from sugarcane bagasse is possible through thermal, chemical or enzymatic hydrolysis [19,27-29]. Suksombat [30] indicated that the hemicellulose content of bagasse was reduced by NaOH treatment. The hydrolysis of the sugarcane bagasse during alkali pretreatment involves solubilization and partial destruction of the hemicellulose to release sugars. As a consequence, the amount of reducing sugar recovered from the bagasse depends on treatment time, temperature and acid/alkali concentration. Hemicellulose is a complex carbohydrate structure that consists of different polymers like pentoses (like xylose and arabinose), hexoses (like mannose, glucose and galactose), and sugar acids. The dominant component of hemicellulose from hardwood and agricultural plants, like grasses and straw, is xylan, while this is glucomannan for softwood [12,31]. Hemicellulose has a lower molecular weight than cellulose, and branches with short lateral chains that consist of different sugars, which are easily hydrolyzable polymers [31]. Hemicellulose serves as a connection between the lignin and the cellulose fibers and gives the whole cellulose–hemicellulose–lignin network more rigidity [32]. The solubilization of hemicellulose compounds into the water starts around 150-180 °C under neutral conditions [33,34]. The solubilization of lignocellulose components not only depends on temperature, but also on other aspects like moisture content and pH [31]. The xylan of hemicellulose can be extracted quite well in an acid or alkaline environment, while glucomannan can hardly be extracted in an acid environment and needs a stronger alkaline environment than xylan to be extracted [31,35,36]. Xylan appears to be the part that can be extracted the most easily. The lignin and hemicelluloses are the most thermal-chemically sensitive [37,38]. During thermal–chemical pretreatment firstly the side groups of hemicellulose react, followed by the hemicellulose backbone [39,40].

In Figures 4 and 5 lignin content and percentage delignification in the sugarcane bagasse was presented at each treatment. Untreated sample was taken as control which consists of 23% lignin content. It was noted after treatment that 2.5% KOH reduces the lignin content upto 7.32% whereas 4% KOH reduces lignin content upto 7.6% (delignification 70.72% and 69.6%) respectively. Figure 6 represents the scanning electron micrograph of the sugarcane bagasse which indicates that raw bagasse was very hard and after treatment holes were created which showed the destruction of tissues. The creation of these holes during treatment was very helpful in the saccharification process which increases the rate of enzymatic hydrolysis reactions. The action of alkaline is to break the ester bonds cross-linking lignin and xylan thus creating pores in the biomass [41]. The enzymatic hydrolysis rate of untreated natural biomass materials was less than 20%, so the materials must be pretreated, during which the cellulose, hemicellulose, and lignin be separated, the cellulase could be easily penetrated to

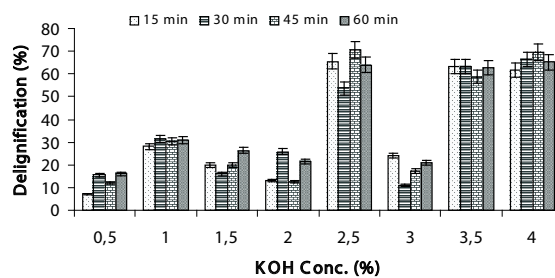


Figure 4. Effect of different concentrations of KOH and steaming time on delignification of sugarcane bagasse

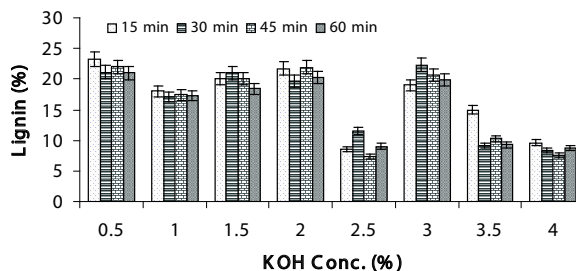


Figure 5. Effect of different concentrations of KOH and steaming time on lignin content of sugarcane bagasse

cellulose, and be hydrolyzed effectively [28]. The major purpose of pretreatment is to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic structural and chemical composition to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars [42]. Zhang and Cai [43] treated rice straw with 2% NaOH and reported reduction in lignin content of before and pretreated straw from 14.9% to 9.5% respectively and also mentioned that after treatment with NaOH the basic tissue become severely shrank. Kim and Lee [44] and Xu *et al.* [45] founded that untreated samples were very hard and pretreated samples were very soft indicating the destruction of the tissues. The removal of lignin is necessary for cellulose to readily become available for the enzymes which permit the yeast to convert glucose into ethanol [46]. Lignin limits the rate of enzymatic hydrolysis by acting as a shield, preventing the digestible parts of the substrate to be hydrolyzed, therefore its removal increases the enzymatic hydrolysis [42]. Lignin normally starts to dissolve into water around 180 °C under neutral conditions [33] and its solubility depends however on the precursor (p-coumaryl, coniferyl, sinapyl alcohol or combinations of them) of the lignin [47]. It is well known that dilute acid pretreatment remove hemicellulose components to expose cellulose for enzymatic digestion and alkaline pretreatment remove lignin and various uronic acid substitutions on hemicellulose that lower the accessibility of enzyme to the hemicellulose and cellulose [48]. According to Kaar and Holtzapple [49] lime pretreatment

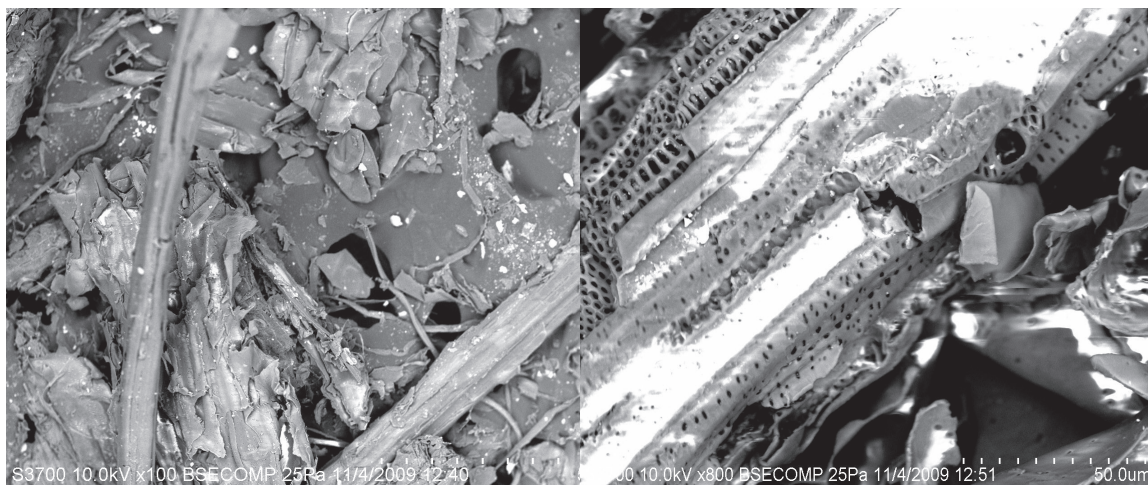


Figure 6 Scanning electron microscopy of sugarcane bagasse fibers. (A) Control, Untreated (magnification, x100) arrows represents the bagasse fibers; (B) Treated with 2.5% KOH (magnification, x800) and the arrows represents the holes in the fibers which indicates the removal of hemicelluloses and lignin.

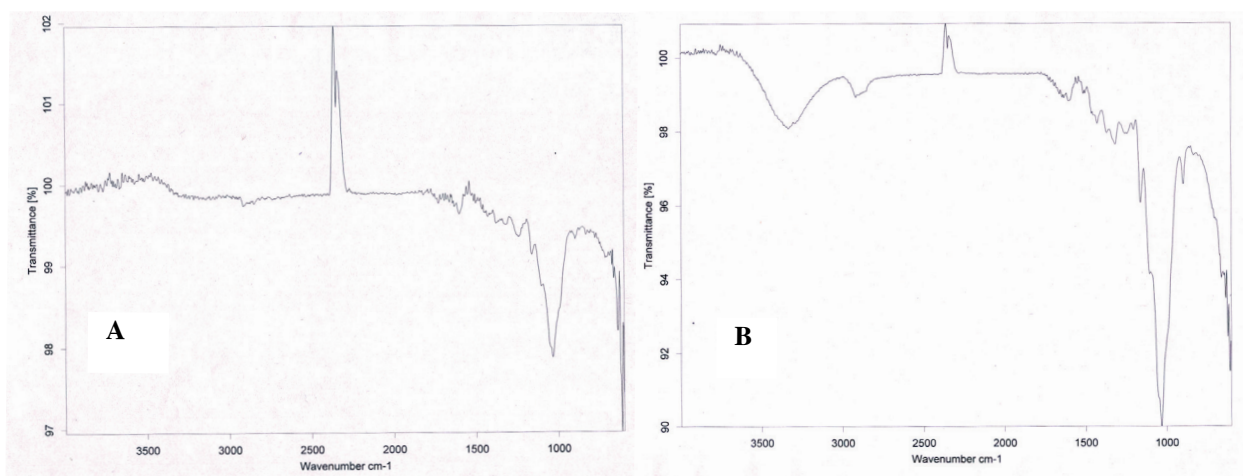


Figure 7. FTIR analysis of sugarcane bagasse fibers. (A) Control, Untreated bagasse fibers; (B) Treated with 2.5% KOH.

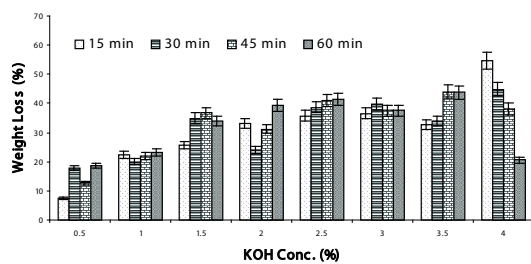


Figure 8. Effect of different conc. of KOH and steaming time on weight loss of sugarcane bagasse after treatment

(with heating) is sufficient to increase the digestibility of low-lignin containing biomass, but not for high lignin containing biomass. Lime pretreatment of switch grass and corn stover did not inhibit the enzymatic saccharification and fermentation steps [50]. The major positive effect of lime is that it is relatively cheap, safe and easily regained from the reaction mixture [51].

Figure 7 shows the spectrum of treated and untreated sugarcane bagasse. The result indicated that alkaline treatment (KOH) causes the degradation of fibrillar structure of cellulose and lignin to greater extent. The absorbance at 3655, 2914, 1601, 1239, 1034, cm^{-1} in Figure 7a are associated with untreated sugarcane bagasse as reported by Sun *et al.* [52]. The band at 3334.2 cm^{-1} is depicting the stretching of hydroxyl group in treated bagasse (Figure 7b). The absorption at 2915 cm^{-1} in treated sugarcane bagasse arises from C-H stretching, moreover the absorbance at 1426, 1315.9, 1161.8 and 1032 cm^{-1} corresponds to the aromatic skeleton vibration, ring breathing in the C-O stretching in lignin [53]. The bands at 1315.92 cm^{-1} are attributed to absorption by C-H and C-O stretching in acetyl group in hemicellulose respectively. The strong band at 1161.88 cm^{-1} in pretreated sugarcane bagasse is assign to C-O stretching in cellulose, hemicellulose and lignin or C-O-C stretching in cellulose and hemicelluloses. The band at 896 cm^{-1} is due to glucosidic linkage [54]. The region of FTIR spectrum between 633 to 606 cm^{-1} was not considered.

During thermochemical treatment some part of the biomass was digested which resulted in weight loss. Figure 8 denotes the weight loss in sample during treatment. Wyman [46] reported that the amount of weight loss during thermochemical treatment was due to lignin removal. Greater the weight loss means greater the lignin removal. Maximum weight loss (57.4%) was noted during treatment of sugarcane bagasse with 4% KOH at 15 min of autoclaving time. By changes the chemical loading and heating time on sugarcane bagasse weight loss varies according to treatment. Similar findings were also reported by Lee *et al.* [55].

Effectiveness of alkaline pretreatment depends on numerous factors but it is generally more effective on agricultural residues and herbaceous crops than on woods. Alkaline pretreatment techniques are basically delignification processes in which significant amount of hemicellulose is solubilized [56,57].

Conclusion

Alkaline treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure. For efficient enzymatic hydrolysis and ethanol production, pretreatment is an important technique which enhances the enzymatic reaction.

Acknowledgement

The authors would like to thank the Ministry of Science and Technology (MoST), Islamabad, Pakistan for the financial support of this work through the project "Production of Bioenergy from Plant Biomass".

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