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Structural and Morphological Alteration of Wheat Straw Biomass Utilizing Alkali Pre-treatment

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ABSTRACT

Through pre-treatment and chemical up-gradation, the three primary lignocellulosic components can be converted into a wide variety of biomass-derived platform fuels, chemicals and materials. In this work, wheat straw was pre-treated using different sodium hydroxide concentrations (1-5%) at 121°C and 15 psi in an autoclave for 60 min. The study revealed that maximum cellulose and delignification were obtained at 2% sodium hydroxide. The content of cellulose (68.39%), delignification (4.62%) and hemicellulose (16.38%) were recorded after alkali pre-treatment of wheat straw biomass. SEM, XRD and FTIR spectroscopy analysis further demonstrated that the alkaline processing of biomass altered its structural and morphological characteristics. The XRD result of the straw powder sample revealed that the crystallinity index improved from 36.80 to 50.64%, indicating higher cellulose content. SEM study indicated the disorderly surface and porosity of pre-treated wheat straw fibers, thus improving cellulose accessibility for cellulose. FTIR showed that some of the peaks missed and others shifted in position due to alkaline pre-treatment, which confirmed the increase in the cellulose content and delignification.

Key words: Wheat straw, alkaline pre-treatment, SEM, XRD, FTIR

INTRODUCTION

Energy production from lignocellulosic wastes is an attractive option since it has the potential to reduce greenhouse gas emissions by as much as 86% when compared to fossil fuels (Kumari and Singh, 2022). Wheat straw (WS) is the second most prevalent biomass feedstock on earth, next to rice straw, and is a readily available carbohydrate-rich source for producing biofuels (Tufail *et al.*, 2021). When it comes to biomass feedstock, WS is second only to rice straw in terms of global abundance; it is also widely available carbohydrate-rich material that may be used to produce biofuels. WS is used in modest amounts for cattle but in enormous quantities for field burning, which contributes to climate change (Tsegaye *et al.*, 2019). Typically, WS is mainly composed of 35 to 45% cellulose, 20 to 30% hemicellulose and 8 to15% lignin which makes it suitable for bioenergy generation (Rani *et al.*, 2022). The covalent cross-links and non-covalent forces hold these three parts together (Tufail *et al.*, 2018). The fundamental drawback of lignocellulosic biomass is its refractory nature owing to its complex structure and inclusion of lignin, which prevents enzymes from accessing hydrolyzable sugars (Millati *et al.*, 2020). Consequently, pre-treatment, including physical, chemical and biological ones or a mix of these, are necessary before using biomass as a substrate. Pre-treatment of the biomass reduces the crystallinity of cellulose and increases surface area to enhance microbial consumption and provide simple sugars for fermentation (Sharma *et al.*, 2022; Ram *et al.*, 2023).

Various physical, chemical and biological pre-treatment stages have been studied by several researchers in the last three decades to increase the rate of fermentable sugars (Saratale *et al.,* 2018). Any pre-treatment method's performance may be assessed using certain criteria, including a reduction in size, energy input minimization and detection of any produced inhibitors. Additionally, the recycling of catalysts, the treatment of waste produced and the recuperation of by-products with added value is also considered. The perfect pre-treatment should be inexpensive, effective for a variety of lignocellulosic substrates, removing the majority of lignin, least number of glucans lose and producing fewer inhibitors (Wang *et al.*, 2015).

Among the chemical methods, sodium hydroxide-based alkali pre-treatment is preferable to acid pre-treatment for maximum cellulose content exposure. The key property of alkaline pre-treatment is that it changes the structure of cellulose fiber, rendering it more thermodynamically stable than the control fiber. After saccharification the pre-treated biomass is used in a fermentation process to produce biofuels (Mohapatra *et al.*, 2017). In the present study, the alkali pre-treatment with sodium hydroxide was applied on WS, and the effect on chemical composition and surface morphology of pre- treated biomass was investigated. Therefore, the main aim of this investigation was to expose cellulose and remove lignin as much as possible from WS following an alkaline pre-treatment. The ester linkages in lignin and hemicelluloses are indeed susceptible to alkaline hydrolysis, which can help in lignin removal and increase cellulose accessibility.

MATERIALS AND METHODS

Wheat straw (*Triticum aestivum*) sample was collected from the field at Sadalpur, a rural area in Hisar district, Haryana. The soil and other contaminants were removed from the samples by washing them with tap water. After that, it was sun-dried to reduce moisture content, and the ground sample was sieved with 80 mm mesh. Finally, it was dried in a hot air oven at 50°C for complete dryness. The completely dried biomass was stored in an air-tight container for further use.

Alkaline pre-treatment was employed to delignify the biomass. Dilute sodium hydroxide (NaOH) of different strengths (1-5% v/ v) were applied to the WS. A solid-to-liquid ratio of 5:100 (w/ v) was used to make sure that the WS powder was completely immersed in the solution. The mixture was kept in an autoclave at 121°C and 15 psi for 60 min. After this, it was allowed to cool. The pre-treated straw sample were filtered from the mixture with muslin cloth to neutralize the residue by washing them with distilled water and continued washing till the pH became neutral (7.0). The pretreated solid was dried at 60°C and grounded to a particle size of approximately 2.0 mm. The characteristics of untreated wheat straw and alkali-pretreated WS, such as Cellulose, Hemicellulose and Lignin, were analyzed by the standard method.

SEM (JSM-7610) was used to examine morphological changes in the untreated and alkali-pretreated WS. Oven-dried and fine-powdered samples were used to determine the morphology of WS. Samples were mounted on the aluminum stubs with the gold coating in a sputter before image analysis. Fourier Transformation Infrared Spectroscopy (FTIR) investigated the various functional groups of the native and alkali-pretreated WS samples. FTIR spectra were taken on a Perkin- Elmer Spectrum. The instrument with the absorption wave range between 400-4000/cm were used for all samples. A trace amount of fine grounded sample was mixed with potassium bromide (KBr) to analyze the various spectral lines. The crystallinity of untreated and alkali- pretreated WS powder was determined by X-ray diffraction (XRD) using X-ray diffractometer with monochromatic CuKα energy basis, having 2θ ranges between 10-50° having phase 0.04 and scanning time of 5 min. To ensure appropriate X-ray exposure, the samples were levelled and placed on the sample holder in fine powder form. The crystallinity index was calculated as:

$$\text{"CrI (\%)}=I_{002}-I_{am}/I_{002}*100\text{"} \quad ....(A)$$

Where,

I_{002}, intensity of the crystalline part of the biomass (e.g. cellulose);

I_{am} the intensity of the amorphous part of biomass (e.g. cellulose, hemicellulose and lignin); I_{002} the lattice peak with the highest diffraction intensity (002); and I_{am} the intensity of amorphous cellulose.

The scattered energy in the amorphous area was constrained at the diffraction angle of (2 theta = 18°), and the diffraction peak at the diffraction angle of 2 theta = 22°.

RESULTS AND DISCUSSION

The percentages of three main components i. e. cellulose, hemicellulose and lignin were investigated in raw WS, and dilute alkali (2%) processed biomass (Table 1). The current research showed that 2% sodium hydroxide yielded the highest levels of cellulose and delignification. Alkali pre-treatment increased the cellulose content in WS from 43.2 to 68.39% by removing amorphous particles from the biomass. Additionally, it also caused cellulose to be more accessible and help in the maximal synthesis of fermentable sugar during enzymatic hydrolysis. The hemicellulose content was reduced from 29.45% in raw WS to 16.38% in pre-treated WS biomass. This may be due to the amorphous structure of the biomass, which was easily hydrolyzed by the diluted sodium hydroxide used in the pre-treatment. However, the lignin content was drastically reduced from 17.68% in raw WS to 4.6% with dilute alkali pre-treatment. Reducing lignin content may greatly enhance enzyme digestibility by making cellulose more accessible to the enzymes. Numerous research related to this study also reported similar results (Asghar *et al.*, 2015; Zheng *et al.*, 2018).

Table 1. Chemical composition of raw WS and dilute alkali pre-treatment of WS

Components (%)	Raw wheat straw	Alkali treated WS (2%)
Cellulose	43.20	68.39
Hemicellulose	29.45	16.38
Lignin	17.68	4.62
Ash	7.40	3.20

Fig. 1 represents the impact of various concentrations of sodium hydroxide (1-5%) on WS and observed the chemical composition of every increasing step of dilute alkali. As dilute sodium hydroxide concentration increased in the pre-treatment samples compared to the raw WS, it was noticed that delignification and cellulose content of biomass increased, whereas hemicellulose content was gradually reduced. Hemicellulose and cellulose interacted to establish a physical barrier that protected against enzyme attack.

An appropriate hemicellulose dissolution rate may potentially improve the digestibility of cellulose and i the effectiveness of enzymatic conversion during hydrolysis. According to the current investigation, maximum cellulose content increased by 58.31%, and hemicellulose reduced by 44.38% at 2% sodium hydroxide, providing the sample with high enzyme digestibility than the others. Cellulase cannot access cellulose due to the physical barrier of lignin, which reduced the enzyme's activity through inefficient binding (Zheng *et al.*, 2018). With 2% NaOH pre-treatment, maximum delignification of 58.31% was achieved, whereas it was 38.50, 15.52 and 11.27%, and 10.90% for the 3, 1, 4, and 5% sodium hydroxide, respectively. Based on the above-mentioned points, 2% alkali pre-treatment was effective in breaking down lignin and hemicelluloses, therefore it was used for further study.

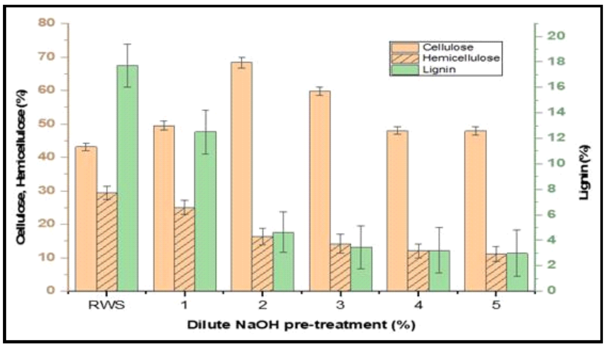

Fig. 1. Composition of raw wheat straw and variation in dry weight after various NaOH pre-treatment.

The reaction of sodium hydroxide with ester bonds, which resulted in the elimination of lignin and the release of cellulose, affected the surface morphology of WS after sodium hydroxide pre-treatment. Fig. 2a shows the rigid, smooth and compact surface structure with densely packed fibers arranged in the bundles. Fig. 2b represents the distorted structure and fiber splitting and formation of scaling (layering) were observed. The outcomes revealed that alkaline pre-treatment caused a hole in the biomass of WS. The lignin and hemicellulose content's disintegration produced these pores. These morphological alterations in the pre-treated biomass showed that these pores offered a surface area for the enzyme cellulase to penetrate and speed up the saccharification process. Dilute alkali pre-treatment reduced the recalcitrant nature of lignocellulosic biomass by dissolving cellulose fiber and increased cellulose accessibility. These findings agreed with those of Asghar *et al.* (2015).

Alteration in chemical composition and functional group of untreated and alkali-treated WS was analyzed using the FTIR analysis (Fig. 3 a,b). The predominated peaks at 3413.12, 2920.18, 1736.38, 1637.36, 1509.03, 1424.21, 1375.94, 1246.97, 1052.84

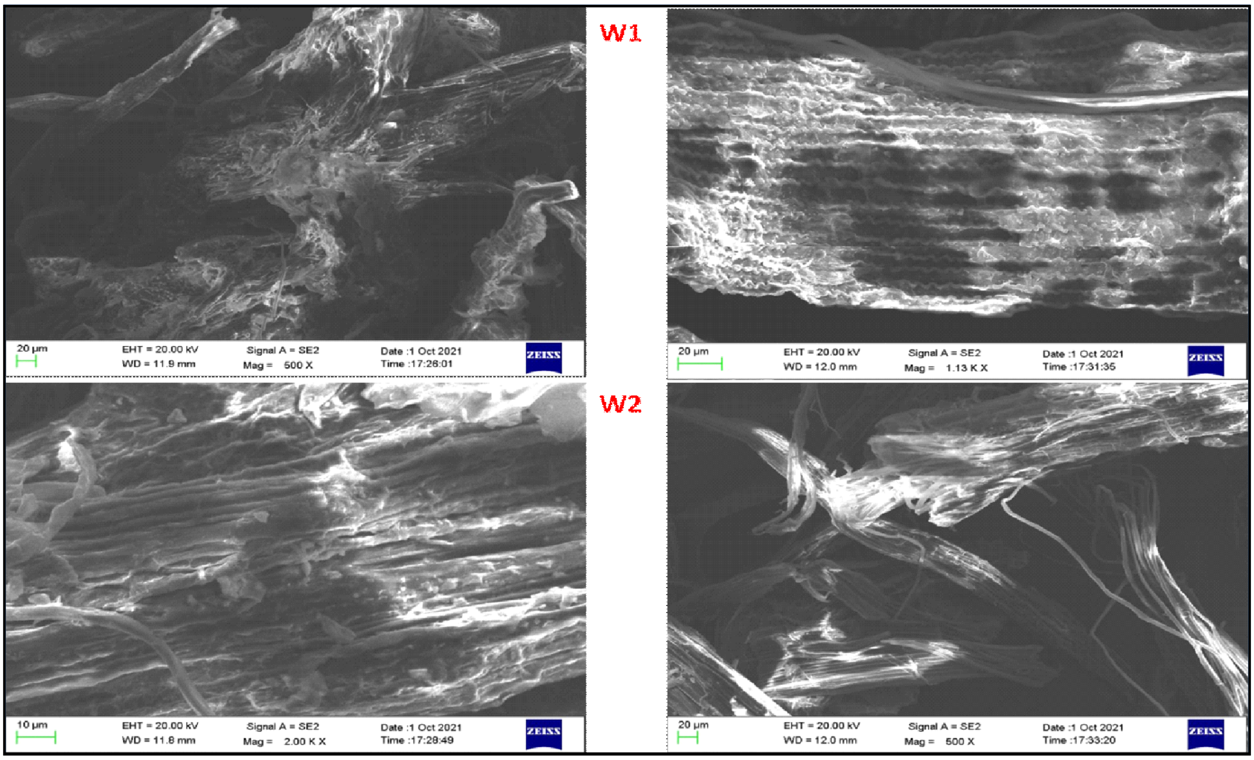

Fig. 2a. SEM images W1: Native wheat straw and 2b. W2: Alkaline pre-treated wheat straw.

and 608.22 in raw WS and peaks at 3431.36, 2920.06, 1637.88, 1422.41, 1374.00, 1163.20, 1029.48, 898.02 and 618.68 were observed in alkali pre-treated WS (Table 2). Consequently, one can see that in the alkali pre-treatment caused some peaks to disappear and others to change positions. Further, as compared to the raw sample, the hydroxyl group content of the biomass decreased, as indicated by a drop in the intensity of the O-H absorption band, because of the alkali pre-treatment. By hydrolyzing ester linkages between lignin and cellulose/hemicellulose, alkali pre-treatment released some cellulose and hemicellulose, which improved the substrate's biodegradability (Wan *et al.*, 2019). A wide band at the peak of 3413.12/cm which was present in raw WS assigned for the hydroxyl group (-OH), and after alkali pre-treatment, this band was extended and appeared at a wavelength of 3431.36/cm. The reduction in the intensity of the (-OH) group confirmed that the 2.0% sodium hydroxide had an impact on the WS. The same wavelength band was also reported by Pankaj *et al.* (2018) work. The band around 2920/cm was attributed to the C-H stretching vibration of the aliphatic chain structure of the lignin, and the same result was observed by Wang *et al.* (2016). The peak at wavelength 1736.38/cm present in raw wheat straw was removed during alkali pre-treatment due to hydrolysis of hemicellulose. The wavelength band at 1509.03 was assigned for C=C stretching of the aromatic ring of lignin which is absent in alkali-pre-treated biomass. The wavelength band peak at 1424.21/cm and 1375.94/cm were assigned for the symmetric CH_2 bending and wagging and C-H bending (Kshirsagar *et al.*, 2015). The sharp stretching and small sharp peak at 1163.20/cm and 889.02/cm assigned for the ß-(1-4)-glucoside linkage appeared due to pre-treatment, indicating that cellulose and hemicellulose contained sugar units. Similar results were observed by Tsegaye *et al.* (2019).

Crystallinity of cellulose offers a qualitative or semi-quantitative evaluation of the amount of amorphous and crystalline cellulosic components in the alkali-pre-treated WS. Complex biomass impacts the crystalline index (CrI), where cellulose is thought to be crystalline, while lignin and hemicellulose are assumed to be amorphous. A significant factor affecting the decomposition of cellulose was recognized as the crystallinity of the biomass material. The key impact of the dilute sodium hydroxide pre-treatment was the elimination of lignin and

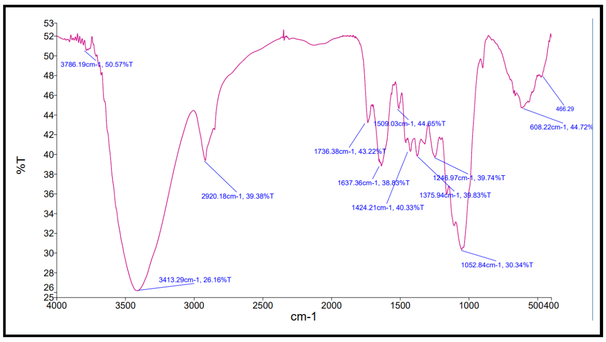

Fig. 3a. FTIR spectrum of raw wheat straw.

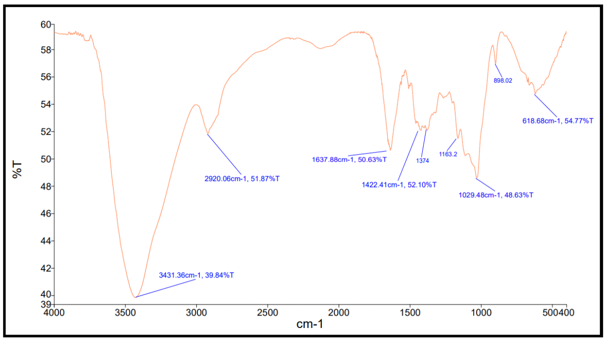

Fig. 3b. FTIR spectrum of alkali pre-treated wheat straw.

hemicellulose, both of which had an amorphous structure. The amorphous portion of the three major components consisted of amorphous cellulose, hemicellulose and lignin (Chen *et al.*, 2020). XRD analysis measured the crystallinity of the total WS sample, comprising lignin and hemicellulose along with amorphous cellulose. After

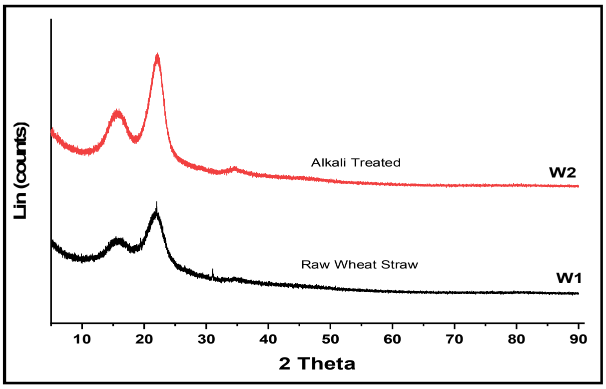

Fig. 4. XRD graph of (W1) : Native wheat straw and (W2) : Alkali pre-treated wheat straw.

extracting the lignin, the pre-treated samples' crystallinity should be increased even though lignin is amorphous while as cellulose is crystalline. In order to analyze the change in the wheat straw's crystalline nature, an X-ray diffraction (XRD) analysis of raw and sodium hydroxide-pre-treated WS was performed (Fig. 4) depicting the diffraction pattern of native and pre-treated WS biomass. The fiber of the WS that had not been treated had a crystalline index of 36.8%, whereas the pretreated WS fiber had 50.64% CrI. After pre-treatment, the enhanced crystallinity index may be the result of glycosidic bond hydrolysis in the exposed cellulose area. Prior investigations also reported a rise in crystallinity index following alkali pre-treatment (Dong *et al.*, 2018; Pankaj *et al.*, 2018; Kininge and Gogate, 2022).

Table 2. Major adsorption bands appeared/disappeared after NaOH pre-treatment

Band assignment	Related structures	Absorption band of raw wheat straw	Absorption appeared or disappeared due to alkali pre-treatment of wheat straw 2% (w/v)
-OH, stretching intramolecular hydrogen bonds	Cellulose-II	3413.12	3431.36
C-H stretching	Lignin	2920.18	2920.06
C=O stretching of acetyl for carboxylic acid	Hemicellulose/Lignin	1736.38	NA
C=C stretching of the aromatic ring	Lignin	1637.36	1637.88
Aromatic C-O stretch	Lignin	1509.03	NA
C-H2 bending	Cellulose	1424.21	1422.41
C-H2 wagging	Cellulose	1375.94	1374.00
C-O adsorption	Cellulose	1246.97	1163.20
C-O stretching	Cellulose	1052.84	1029.48
Asym. out of phase ring stretching (Cellulose)	Cellulose/ Hemicellulose	NA	898.02
C-H bending	Hemicellulose	608.22	618.68

CONCLUSION

It was observed that wheat straw (WS) had a considerable amount of cellulose (43.20%), hemicellulose (29.45%) and lignin (17.68%) component, showing that it was a promising source of carbohydrate that may be further transformed into monomeric sugars. Alkali pre-treatment with 2% sodium hydroxide enhanced the cellulose content (68.39%), whereas it reduced the hemicellulose (16.38%) and lignin (4.62%) content. It was evident from the decrease in hemicellulose and lignin that the alkaline pre-treatment increased the surface area available for enzymatic hydrolysis. SEM pictures clearly revealed that alkali pre-treated biomass had much more destroyed structures than raw WS. XRD analysis indicated that the crystallinity index of pre-treated WS was higher (50.64%) as compared to raw WS (36.80%). FTIR results showed that the crystallinity of pre-treated wheat straw biomass was slightly enhanced as compared to native WS.

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