

Structural Modification of Sugarcane Bagasse using Alkaline, Acid, and Bleaching Pretreatments for Enhanced Yield of Poly (1→4)-β-D-glucopyranose (Cellulose) and Hemicellulose for Utilization as Biocomposites

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Abstract

Sugarcane bagasse, a lignocellulosic byproduct generated in substantial quantities by the sugar industry, represents a promising feedstock for the sustainable production of enzymes and bio-based chemicals. Its high (1→4)-β-D-glucopyranose (cellulose) content, abundant availability, and low cost make it an ideal candidate for bioconversion processes. However, the inherent recalcitrance of bagasse, primarily due to its lignin-rich matrix, necessitates effective pretreatment strategies to enhance enzymatic digestibility. This study presents a comparative investigation of three distinct pretreatment methods—alkaline, acid, and bleaching—applied to sugarcane bagasse, with the goal of improving its structural accessibility and compositional suitability for downstream bioconversion. Pretreatments were conducted using sodium hydroxide (NaOH), sulfuric acid (H₂SO₄), and sodium hypochlorite (NaOCl)-based bleaching under controlled conditions. Each method was evaluated for its effectiveness in delignification, hemicellulose removal, and (1→4)-β-D-glucopyranose (cellulose) exposure. The physicochemical transformations induced by these pretreatments were characterized using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Thermogravimetric Analysis (TGA). Result revealed that alkaline pretreatment with 1N NaOH achieved the highest degree of delignification (7.16% residual lignin), while acid and bleaching treatments offered complementary advantages in structural disruption and thermal behavior. This comparative analysis highlights the distinct mechanisms and efficiencies of each pretreatment strategy, providing valuable insights into optimizing sugarcane bagasse for enzymatic hydrolysis and bioprocessing applications. The findings contribute to the development of more efficient and sustainable biomass pretreatment workflows for cellulase enzyme production and other biotechnological uses.

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INTRODUCTION

The transition to renewable energy sources has become a global priority due to growing concerns about climate change, energy security, and the depletion of fossil fuel reserves. Among the various alternatives, biofuels have emerged as a promising solution for reducing greenhouse gas emissions and meeting the world's increasing energy demand. The production of second-generation biofuels, derived from lignocellulosic biomass, offers a sustainable

pathway by utilizing agricultural residues, forestry waste, and other non-food feedstocks. This approach addresses the limitations of first-generation biofuels, which rely on food crops like corn and sugarcane and raise ethical concerns regarding food security and land use competition [1]. Sugarcane bagasse, the fibrous byproduct of sugar production, is one of the most abundant and economically viable lignocellulosic feedstocks available. Composed primarily of (1→4)-β-D-glucopyranose (cellulose), hemicellulose, and lignin, sugarcane bagasse holds significant potential for bioconversion into biofuels and value-added biochemicals [2]. However, its complex structural composition presents considerable challenges for enzymatic hydrolysis and microbial fermentation. The dense lignocellulosic matrix, reinforced by lignin, restricts access to fermentable sugars, thereby reducing process efficiency and increasing production costs [3]. Effective pretreatment methods are critical to overcoming these challenges, as they enhance the digestibility of lignocellulosic biomass by disrupting its intricate structure. Among the various pretreatment strategies—physical, chemical, biological, and physicochemical—alkaline pretreatment has gained significant attention due to its efficiency and relative simplicity [4]. This method involves the application of alkaline agents, such as sodium hydroxide (NaOH), to selectively remove lignin, reduce cellulose crystallinity, and increase the porosity of the biomass. These structural modifications improve enzymatic access to cellulose and hemicellulose, enhancing the overall efficiency of bioconversion processes [5].

Alkaline pretreatment offers several advantages over other methods, including selective delignification, reduced carbohydrate loss, and operation under milder conditions, which minimizes energy consumption and environmental impact [6]. Additionally, this technique is particularly well-suited for large-scale applications due to its scalability and cost-effectiveness. However, optimizing pretreatment parameters, such as chemical concentration, reaction time, and temperature [7] is essential to maximize delignification while preserving the integrity of fermentable sugars. Various pretreatment strategies have been developed for the delignification of biomass, including physical, chemical, biological, and hybrid approaches such as ammonia fiber expansion and steam explosion. Each method has its own advantages and limitations. For instance, physical methods like milling and grinding reduce the need for chemicals but are energy-intensive, especially on an industrial scale [8]. Biological pretreatments can break down lignin and hemicellulose effectively, yet they require a prolonged processing time [9]. Chemical pretreatments are widely researched and can be efficient, but often generate hazardous by-products like hydrogen sulfide. Dilute acid treatments effectively remove hemicellulose but are corrosive and environmentally damaging. In contrast, alkaline pretreatments—using agents such as sodium hydroxide (NaOH), lime, ammonia, or alkaline hydrogen peroxide (AHP)—are generally more suitable for enzymatic hydrolysis because they efficiently eliminate lignin with minimal carbohydrate loss. Among these, hydrogen peroxide is commonly used in the pulp and paper industry for its bleaching capabilities. Under alkaline conditions (pH ~11.5), hydrogen peroxide generates hydroperoxyl anions that promote the breakdown of lignin and hemicellulose. Significant research has focused on optimizing AHP methods to improve enzymatic conversion of lignocellulosic biomass. Many researchers reported successful separation of 38.7% hemicellulose and 75.4% lignin from corn cobs after a 6-hour AHP treatment. In this study, we present a streamlined five-step process for cellulose extraction from sugarcane bagasse (SCB), emphasizing both efficiency and environmental sustainability. Beyond its utility in enzyme production, the high-purity cellulose obtained from SCB also holds significant potential in the field of biopolymer composites. When integrated into polymer matrices, cellulose acts as a natural fiber reinforcement that improves the mechanical and thermal properties of bio-based plastics. This dual application that is bioconversion and material engineering underscore the versatility of sugarcane bagasse-derived cellulose in circular bioeconomy frameworks.

MATERIALS AND METHODS

Preparation of Sugarcane Bagasse

The study utilized sugarcane bagasse sourced from a local market in Kanpur. The raw biomass underwent a systematic preparation process that included comprehensive washing to remove surface contaminants, thorough dehydration, and precision milling to achieve a standardized 40-mesh particle size [10].

Alkaline Pretreatment

For the experiments, 40 grams of bagasse were processed in a 500 ml reaction vessel containing 500 ml of NaOH solution. Two NaOH concentrations of 1N and 2N were tested with treatment duration of 30 minutes. The experiments were conducted at 100°C under 1 bar pressure [11]. Compositional analysis was performed using the Chesson analytical protocol, which involved sequential extraction steps. Initial water extraction was carried out by refluxing a 1-gram sample with 150 ml of distilled water at 100°C for 60 minutes, followed by filtration, thorough washing with heated water, and oven-drying for consistent mass determination. Dilute sulfuric acid treatment involved exposing the residual material to 1N sulfuric acid at 100°C for 60 minutes, neutralizing the sample through washing, and recording the mass. Concentrated sulfuric acid modification included macerating the material with 10 ml of 72% sulfuric acid at ambient temperature for 4 hours, followed by secondary acid reflux, neutralization, and final mass measurement after thermal processing [12]. This methodology enabled a detailed characterization of structural modifications induced by alkaline pretreatment [13,14]. The analysis focused on quantifying changes in lignin, cellulose, and hemicellulose content, providing insights into the compositional and structural transformations of sugarcane bagasse as discussed in Table 1.

Acid Pretreatment

Acid pretreatment is a commonly employed technique to enhance the digestibility of lignocellulosic materials like sugarcane bagasse (SCB) by targeting hemicellulose removal and partially disrupting the lignin structure. Initially, SCB is thoroughly washed with distilled water to remove dirt and soluble impurities, then oven-dried at 60–70°C to a constant weight. The dried material is ground or milled to a uniform particle size, typically between 0.5 mm and 2 mm, to increase surface area and improve treatment efficiency [15]. For the pretreatment, the milled SCB is mixed with a dilute acid solution—most frequently sulfuric acid (H_2SO_4) at a concentration of 1–2% (w/v)—in a solid-to-liquid ratio of approximately 1:10 (g/mL). This mixture is then subjected to thermal treatment, typically in a pressurized reactor or autoclave at temperatures ranging from 120°C to 160°C for 30 to 60 minutes. The acid catalyzes the hydrolysis of hemicellulose into fermentable sugars, while also loosening the lignin-cellulose matrix. After the reaction, the mixture is cooled to room temperature and filtered to separate the liquid hydrolysate from the solid cellulose-rich residue. The solid portion is washed thoroughly with distilled water until neutral pH is achieved to eliminate residual acid and inhibitory byproducts. This cellulose-enriched SCB is then ready for enzymatic hydrolysis or further processing.

Bleaching Pretreatment

The bleaching pretreatment of sugarcane bagasse (SCB) using sodium hypochlorite (NaOCl) is an effective method to remove residual lignin and improve the whiteness and purity of cellulose. The process begins with the preparation of SCB, which is thoroughly washed with distilled water to eliminate dirt and soluble impurities, followed by oven-drying at 60–70°C until a constant weight is achieved. The dried material is then ground to a fine particle size (typically <2 mm) to increase surface area [16]. Prior to bleaching, the SCB is often subjected to an alkaline treatment with NaOH (2–5% w/v) at 80–90°C for 1–2 hours to partially remove hemicellulose and loosen the lignin structure. After alkaline treatment, the biomass is filtered and rinsed with distilled water until a neutral pH is obtained. For the bleaching step, the solid residue is immersed in a sodium hypochlorite solution, typically at a concentration of 1–5% (v/v), and the pH is maintained in the alkaline range (around pH 10–11) to enhance oxidative activity. The reaction is conducted at 60–80°C for 1–2 hours under continuous stirring. Sodium hypochlorite acts as an oxidizing agent, breaking down lignin and enhancing cellulose brightness. Following bleaching, the material is thoroughly washed with distilled water until neutral and then dried [17, 18]. This treatment results in a cellulose-rich material with reduced lignin content, suitable for further processing such as enzymatic hydrolysis or composite reinforcement.

RESULTS AND DISCUSSION

One of the crucial phases in the study of bioethanol is alkaline pretreatment, which is accomplished by boiling with sodium hydroxide. The chemical makeup of sugarcane bagasse was examined both before and after the treatment, and pretreatment cooking was carried out. The results are presented in Table 2.

Table 1. Comparison of biomass pretreatment methods.

Pretreatment type	Description	Advantages	Disadvantages
Acid Pretreatment	Uses dilute acids (e.g., H ₂ SO ₄) to hydrolyze hemicellulose and disrupt biomass structure.	- Removes hemicellulose effectively - Enhances enzymatic accessibility - Short reaction time	- Corrosive to equipment - Forms inhibitory by-products (e.g., furfural) - Requires neutralization
Alkaline Pretreatment	Uses alkalis (e.g., NaOH, ammonia) to remove lignin and swell cellulose fibers.	- Efficient lignin removal - Increases digestibility - Minimizes sugar loss - Increase cellulose purity	- Longer treatment time - Produces alkaline wastewater - Chemical recovery may be needed
Bleaching Pretreatment	Uses oxidizing agents (e.g., NaOCl) to degrade and remove lignin post-alkaline treatment.	- Improves whiteness - Enhances thermal/stability properties	- Risk of cellulose degradation - Chemical handling issues - Environmental impact

Table 2. Chemical composition percentages of sugarcane bagasse.

Treatment	Heating time (min)	Hemicellulose (%)	Cellulose (%)	Lignin (%)	Other components (<i>extractives, ash, moisture, etc.</i>) (%)
Untreated	0	22.90 ± 0.67	40.33 ± 0.86	17.50 ± 0.65	19.27
NaOH 1N	30	17.06 ± 0.40	62.10 ± 0.13	8.34 ± 0.35	12.50
NaOH 2N	30	20.86 ± 0.90	56.33 ± 0.79	9.93 ± 0.57	12.88

Based on Table 2, untreated sugarcane bagasse (without pretreatment) contained 40.33% cellulose and 17.50% lignin. After delignification with 1N NaOH for 15 minutes, the cellulose content increased to 62.10%, representing a 46.9% increase, while lignin decreased by 39.6%. In contrast, delignification with 2N NaOH for 30 minutes resulted in the lowest cellulose content and the highest lignin content compared to the other treatments. Specifically, this process increased cellulose by 26.8% and reduced lignin by 43.3%. These results indicate a general trend where higher cellulose content correlates with greater lignin reduction, except for the 15-minute treatment with 1N NaOH. This anomaly might be due to a stronger chemical linkage between lignin and cellulose, as well as the crystalline structure of cellulose at this stage. During the 15-minute delignification, cellulose content was high, but lignin levels remained elevated, possibly because only a small fraction of lignin transitioned into the liquid phase, while most cellulose remained in the solid phase. From this analysis, delignification with 1N NaOH for 30 minutes appears to be the most effective treatment, as it achieved the highest reduction in lignin content. Additionally, 1N NaOH was more effective than 2N NaOH in increasing cellulose content and reducing lignin in sugarcane bagasse.

Impact of Alkaline, Acid, and Bleaching Pretreatments on the Chemical Composition of Sugarcane Bagasse

Sugarcane bagasse (SCB), the fibrous byproduct generated during sugarcane processing, is primarily composed of cellulose, hemicellulose, and lignin. These structural components are intricately linked within a rigid lignocellulosic framework, making them resistant to direct utilization. To improve the availability of cellulose and hemicellulose for downstream processes such as enzymatic hydrolysis or biopolymer production, various pretreatment methods are applied to break down this complex matrix.

Table 3. Comparison of cellulose and hemicellulose content before and after alkaline pretreatment.

Sample type	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Other components (%)
Raw SCB (untreated)	38.0	27.5	21.0	13.5
After Alkaline Treatment	54.0	25.0	8.5	12.5
After Acid Treatment	42.0	10.5	19.5	28.0
After Bleaching	63.5	8.0	4.5	24.0

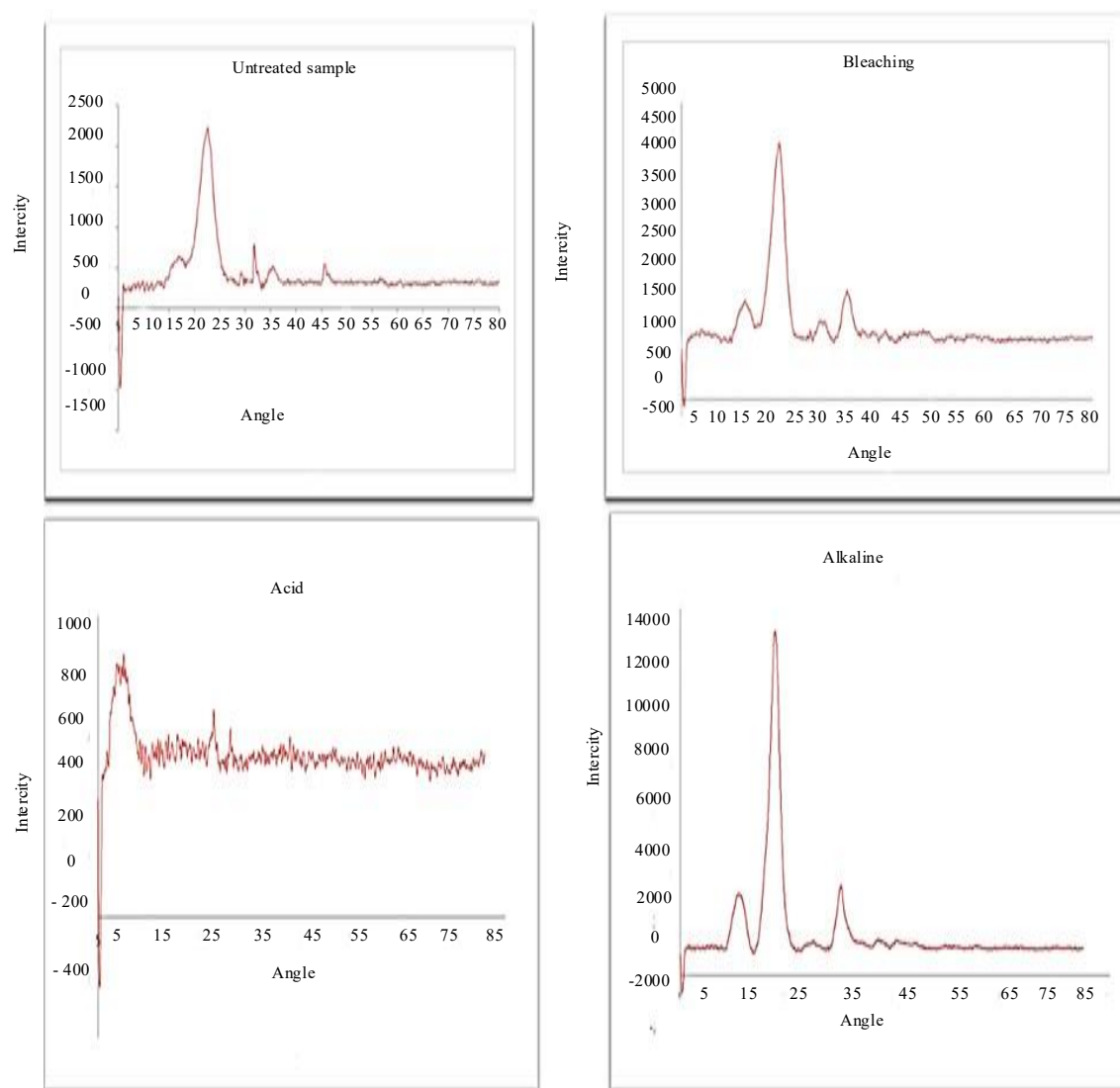
**Figure 1.** Diffractogram of Untreated bagasse; bleaching; acid and alkaline treated sugarcane bagasse.

Table 3 illustrates the compositional changes in sugarcane bagasse (SCB) before and after various pretreatment processes. The untreated SCB comprised 38.0% cellulose, 27.5% hemicellulose, and 21.0% lignin, with the remaining 13.5% consisting of extractives, ash, moisture, and other minor constituents. Notable alterations in cellulose and hemicellulose content were observed following alkaline, acid, and bleaching treatments. Alkaline pretreatment, using sodium hydroxide (NaOH), significantly increased cellulose content to 54.0%. This enhancement is primarily due to the effective delignification, as lignin content decreased from 21.0% to 8.5%. Hemicellulose was also partially removed, lowering its content to 25.0%. These results suggest that alkaline treatment efficiently breaks down the lignin-carbohydrate matrix, thereby improving cellulose accessibility and concentration. Acid pretreatment led to a substantial reduction in hemicellulose, which decreased to 10.5%, while cellulose

content showed a modest increase to 42.0%. The selective hydrolysis of hemicellulose under acidic conditions explains this trend. However, this method was less effective in lignin removal, with 19.5% still remaining. Additionally, an increased percentage of undefined components (28.0%) indicates the formation of degradation byproducts and possible sugar losses during the process. The bleaching treatment was the most efficient in purifying cellulose, elevating its content to 63.5% while significantly reducing lignin to 4.5% and hemicellulose to 8.0%. This demonstrates the strong oxidative action of the bleaching agents in removing both lignin and residual hemicellulose, yielding a highly cellulose-rich substrate. Such material is particularly suitable for enzymatic hydrolysis or reinforcement in bio-composite fabrication. In summary, all pretreatment methods altered the chemical profile of SCB to varying degrees. Alkaline and bleaching treatments were most effective in enriching findings emphasize the need to tailor pretreatment strategies according to the desired downstream application.

XRD Analysis

Based on Figure 1, it is observed that untreated bagasse exhibits peaks at $2\theta = 23^\circ$ and $2\theta = 15^\circ$, corresponding to cellulose. The height of these peaks indicates a relatively high level of cellulose crystallinity. During the process, the cellulose chains expand as the base diffuses into the crystalline regions, leading to rearrangements and structural damage in the crystalline cellulose. The crystallinity index (I_c) of untreated bagasse was determined to be 20.59%, while bagasse after delignification had an I_c of 13.33%. This indicates a 35.3% reduction in crystallinity. The XRD profiles of untreated and chemically treated sugarcane bagasse exhibit noticeable variations in crystallinity and structural composition.

In the untreated sample, a moderate diffraction peak appears near 22° , which is characteristic of crystalline cellulose surrounded by a largely amorphous matrix made up of lignin and hemicellulose. The acid-pretreated sample presents a wider and less intense peak, indicating a more disordered structure caused by partial removal of hemicellulose, though much of the lignin remains. The bleached sample shows clearer and sharper peaks, particularly around 22° , reflecting an increase in crystallinity due to more effective elimination of amorphous substances such as lignin and hemicellulose. Among all, the alkaline-treated sample displays the highest peak intensity, signifying superior cellulose crystallinity. This implies that alkaline treatment most effectively enhances cellulose purity and structural alignment by eliminating non-cellulosic components. XRD analysis confirms that the type of chemical pretreatment greatly affects the crystalline nature and internal order of the biomass. This structural disruption transforms crystalline cellulose into a more amorphous form, resulting in a lower crystallinity index. The crystallinity of cellulose in this diffractogram was calculated using Equation 1.

$$I_c = \frac{I_{200} - I_{am}}{I_{200}} \times 100 \quad (\text{Eq. 1})$$

where,

- I_c = Crystallinity Index
- I_{200} = Peak intensity at $2\theta = 23^\circ$
- I_{am} = Peak intensity between $2\theta = 23^\circ$ and $2\theta = 15^\circ$

This implies that alkaline treatment most effectively enhances cellulose purity and structural alignment by eliminating non-cellulosic components. XRD analysis confirms that the type of pretreatment greatly affects the crystalline nature and internal order of the biomass.

FTIR Analysis

FTIR spectroscopy was employed in this study as it offers a straightforward approach to analyzing chemical changes occurring during NaOH-based delignification. Infrared spectroscopy is utilized to identify functional groups in compounds and to assess chemical structural changes in lignocellulosic materials. The results are presented in Figure 2.

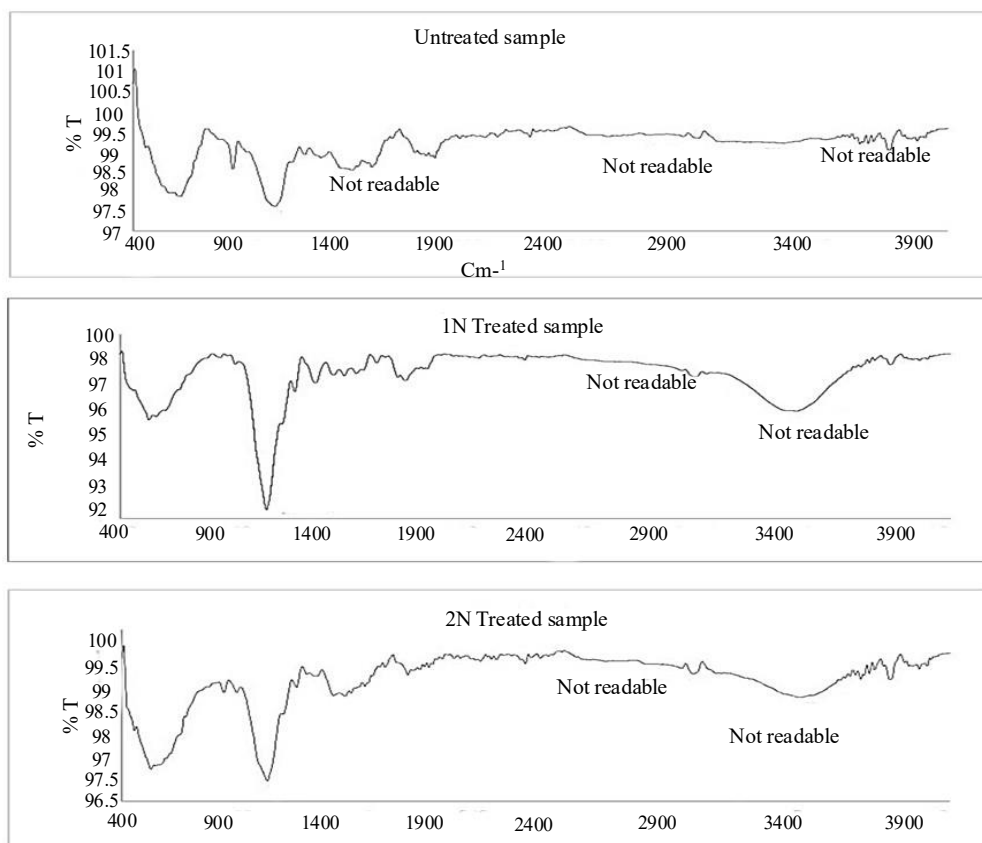


Figure 2. IR spectra of untreated bagasse; IR Spectra after pretreatment with NaOH 1N; IR Spectra after pretreatment with NaOH 2N.

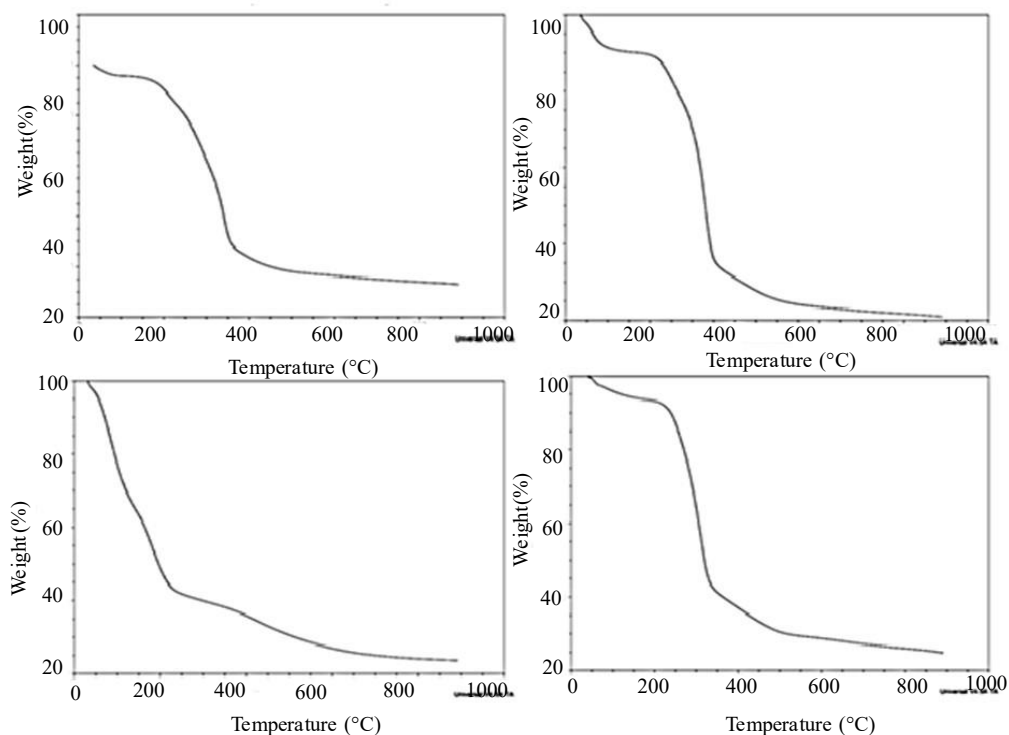


Figure 3. The thermogravimetric analysis of Untreated bagasse; bleaching; acid and alkaline treated sugarcane bagasse.

As shown in Figure 2, a clear distinction is evident between the spectra of untreated bagasse and those subjected to NaOH delignification, highlighting the structural changes brought about by the alkaline treatment. The spectra showed a broad peak within the range of 3332–3505 cm^{-1} , associated with -OH functional groups, and a peak near 2888.3 cm^{-1} , corresponding to CH stretching. Following delignification, a peak appeared around 1034.52 cm^{-1} , whereas untreated bagasse displayed a peak at approximately 1031.9 cm^{-1} , both attributed to C-O-C stretching of β -(1 $\rightarrow$ 4) glycosidic bonds [19,20].

Furthermore, the peak at 2883 cm^{-1} in the fresh bagasse, representing -CH₂ stretching, shifted to 2881.3 cm^{-1} after delignification. An increased intensity of peaks between 900–1100 cm^{-1} indicated a higher cellulose content in the treated material. These spectral changes confirm that delignification successfully enhanced the cellulose content in the NaOH-treated bagasse.

TGA Analysis

Thermogravimetric analysis (TGA) shown in Figure 3, revealed distinct degradation phases in cellulose, reflecting the varying thermal stability of its individual components. The thermal decomposition profiles of untreated and pretreated sugarcane bagasse displayed notable differences, influenced by the specific pretreatment techniques applied. In the untreated sample, initial weight loss occurs between 30–100°C due to moisture evaporation, followed by major thermal decomposition between 250–350°C, primarily due to the breakdown of hemicellulose and cellulose. This sample retains a high residual mass, mainly from lignin and mineral content. Acid-treated bagasse shows a comparable moisture loss range but decomposes at slightly higher temperatures, suggesting partial removal of hemicellulose and some lignin, leading to modest improvements in thermal stability [21,22]. Bleached bagasse displays the highest thermal stability, with a more pronounced shift to higher degradation temperatures and the lowest residue, indicating efficient removal of lignin and hemicellulose. However, excessive treatment may risk degrading some cellulose. Alkaline-treated bagasse shows a broader decomposition curve at elevated temperatures, reflecting effective lignin removal and preservation of cellulose, offering a balanced combination of structural integrity and thermal resilience. The observed thermal behavior can be attributed to the pyrolytic breakdown of the cellulose acetate polymer chain, which is followed by deacetylation.

Additionally, the pretreatment process facilitates the removal of less thermally stable components such as lignin and hemicellulose from sugarcane bagasse (SCB) [23,24]. This is primarily due to the reduced presence of hemicellulose and lignin resulting from the dewaxing pretreatment.

CONCLUSION

This research demonstrates that chemical pretreatment significantly enhances the suitability of sugarcane bagasse as a substrate for cellulase enzyme production. Among the different pretreatment techniques examined—alkaline, acid, and bleaching—the alkaline method proved to be the most efficient, achieving substantial lignin removal and greater cellulose accessibility. This conclusion is supported by various structural, morphological, and compositional analyses. While acid pretreatment was effective in removing hemicellulose, it was less successful in reducing lignin content. Bleaching, on the other hand, facilitated notable lignin removal but offered only moderate improvement in enzymatic digestibility. Advanced characterization tools such as FTIR, TGA, XRD, and compositional profiling revealed that each pretreatment uniquely modifies the biomass's physicochemical properties, influencing its effectiveness for cellulase production. TGA and XRD analyses confirmed that the extracted cellulose exhibits strong thermal stability and a high crystallinity index. These results indicate that the applied treatment methods were effective in achieving the desired structural properties of cellulose. Owing to its improved thermal resistance and crystalline structure, the cellulose obtained from sugarcane bagasse presents strong potential as a reinforcing material in eco-friendly polymer matrices like polylactic acid (PLA), polyhydroxyalkanoates (PHA), or starch-derived polymers. Such cellulose-based biocomposites are gaining attention for use in sectors like packaging, automotive, and healthcare because of their environmentally friendly nature, biodegradability, and enhanced mechanical performance.

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