

Fractionation of cellulose and lignin from sugarcane bagasse via the alkaline and acid chemical process

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Abstract. This research aimed to fractionate cellulose and lignin from sugarcane bagasse (SCB) using the alkaline and acid chemical process. Sulphuric acid (H₂SO₄) and Sodium hydroxide (NaOH) were selected as the pretreatment chemical solvents. After pretreatment, the cellulose content and lignin were 98.5% and 79.2%, respectively. Fourier Transform Infrared (FTIR) Spectroscopy, ¹H and ¹³C Nuclear Magnetic Resonance (NMR), and Scanning Electron Microscopy (SEM) were employed to analyse the material. Raw SCB shows the vibration absorption peaks of O-H and C-H stretching at 338.23 and 2897.37 cm⁻¹, which are correlated with cellulose, hemicellulose, and lignin molecules. The vibration of O-H and C-H functional groups was observed on extracted cellulose and lignin biopolymers. However, the intensity of vibration peaks decreased, especially for lignin biopolymer. The absorption band observed at 1603.74 and 1541 cm⁻¹ was attributed to the aromatic (C=O, C=C) structure. ¹H and ¹³C NMR spectra detected the presence of both the aromatic regions and the sidechain regions on raw SCB and SCB-lignin. However, only the sidechain regions were detected on SCB-cellulose. SEM analysis reveals that the surfaces of raw SCB, SCB-cellulose, and SCB-lignin exhibit smooth, irregular, and uneven shapes, respectively. The results show that cellulose and lignin were simultaneously recovered from SCB.

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1 Introduction

Over the years, fossil fuels have been the central backbone of a country's economy, as they are utilised in numerous applications, particularly in the energy sector [1]. The disadvantage of fossil fuels is that they are produced from non-renewable resources [2, 3, 4]. Researchers and industries have devoted themselves to finding alternative materials that could replace fossil fuels [5]. Biomass is regarded as a material that has the potential to replace non-renewable products, such as fossil fuels [6]. The advantage of biomass is not only its sustainability but also its environmental friendliness [7, 8]. Plant biomass is often referred to as lignocellulosic biomass because it primarily consists of three major components: cellulose, hemicellulose, and lignin [9, 10]. These biopolymers can be converted into valuable products, such as biofuels [11, 12], biochemicals [11], and biomaterials [13]. However, the pretreatment step is required to fractionate these valuable biopolymers. Physical and chemical methods are often employed to extract the lignocellulosic biopolymers [14]. However, the latter is frequently used because it is more effective [15]. More often, during the pretreatment of lignocellulosic biomass, researchers are more interested in recovering a single biopolymer of interest, especially cellulose [14]. Vaz et al. [14] reported pretreatment of SCB, but only cellulose was collected and further hydrolysed with an enzyme. In another study, Zhu et al. [15] pretreated SCB to enhance lignin removal and improve sugar release during enzyme hydrolysis. However, recently, researchers have devoted themselves to converting hemicellulose and lignin into valuable products [16, 17]. For example, Wang et al. [16] recovered all three major biopolymers from corn stalk and converted them into valuable products such as furfural, antioxidant and ethanol. Nevertheless, few studies have focused on recovering two or all three biopolymers. Recovering all biopolymers will not only add value to hemicellulose and lignin but also reduce the amount of waste generated in the environment. Herein, the focus was on fractionating cellulose and lignin from raw sugarcane bagasse using an acid and alkaline chemical method. Chemicals such as sulphuric acid and sodium hydroxide are often employed during the pretreatment of lignocellulosic biomass [15]. Sulphuric acid has the potential to hydrolyse the bonds between biopolymers such as cellulose and hemicellulose [18]. At the same time, the sodium hydroxide can solubilise the lignin biopolymer [18]. The primary advantage of this method over other conventional pretreatment methods, such as organosolv and ionic liquids, is its cost-effectiveness. The disadvantage is that it is less environmentally friendly compared to other methods. Therefore, after the pretreatment, careful disposal of wastewater is recommended. The pretreatment of lignocellulosic biomass, like sugarcane bagasse, has been reported before [19, 20]. However, to the best of our knowledge, no study has focused on recovering both cellulose and lignin simultaneously from sugarcane bagasse using both acidic (i.e., sulphuric

acid) and alkaline (i.e., sodium hydroxide) chemical methods and investigating their physicochemical properties. The objective was to chemically pretreat sugarcane bagasse with diluted sulfuric acid and sodium hydroxide to fractionate cellulose and lignin. To evaluate the physicochemical properties of the recovered cellulose and lignin using characterisation or analytical techniques such as FTIR, ^1H and ^{13}C NMR and SEM.

1.1 Materials and Methods

1.1.1 Collection of the materials

Sugarcane bagasse (SCB) was collected after the juice was extracted from sugarcane grown in Thohoyandou, Limpopo Province, South Africa. The SCB was sun-dried before it was milled into a smaller particle size. Then, it was washed with distilled water to remove dust and other unwanted materials. SCB was again dried in the oven before undergoing the pretreatment steps. All the chemicals were purchased from Sigma and were of analytical grade.

1.1.2 Extraction of cellulose

The fractionation of cellulose and lignin from raw SCB is summarised in Figure 1. Dried SCB was milled into fine powders using an electric blender. A certain amount of milled raw SCB was transferred into a beaker containing a mixture of ethanol and distilled water (1:1 ratio), and it was stirred at 1000 rpm for 1 h at 50 °C. This was done to remove the wax chemicals and other impurities. Subsequently, the dewaxed SCB was pretreated with 5% H_2SO_4 (v/v) for 30 min at 100 °C at a solid-to-liquid ratio of 1:10. The mixture was then filtered using a vacuum filter. The solid material was collected and pretreated with 10% NaOH (w/v) for 1 h at 100 °C, using a solid-to-liquid ratio of 1:10. The mixture solution was then filtered using a vacuum filter. The remaining solid (i.e., cellulose) was bleached with sodium hypochlorite and acetic acid (1:1 ratio) for 2 h at 80 °C. The extracted cellulose was denoted SCB-Cellulose. All the experiments were done in duplicate.

1.1.3 Lignin Recovery

The filtrate (i.e., black liquor) was collected for lignin recovery. H_2SO_4 (20%, v/v) was slowly added into the filtrate to precipitate the lignin with magnetic stirring for 30 min. After adding the acid, the solution changed from black to brown, and gradually, the precipitate started to form. The solution containing the precipitate was centrifuged at 4000 rpm for approximately 10 min to separate the precipitate from the liquid. The recovered precipitate was dried in an oven at 100 °C for approximately 2 h. Moreover, the recovered lignin was denoted SCB-lignin.

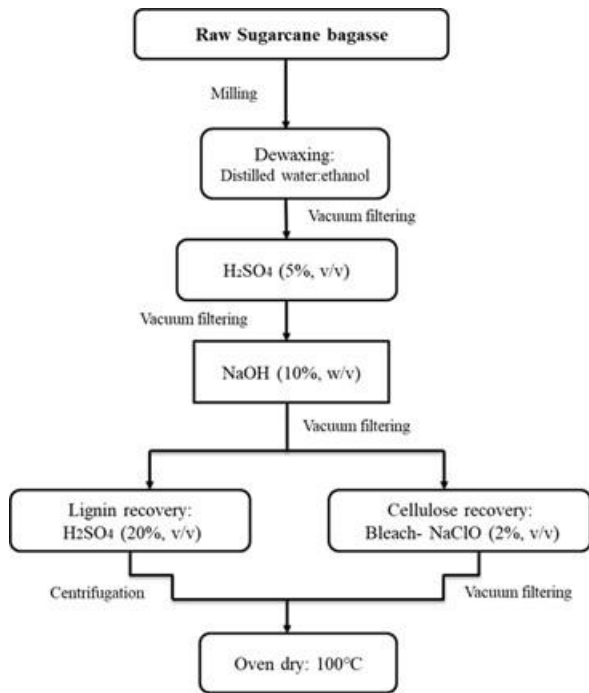


Fig. 1. Fractionation of cellulose and lignin from sugarcane bagasse using acid and alkaline chemicals.

1.2 Characterisation of materials

The functional groups of the raw SCB and its derivatives were analysed using the FTIR technique. The FTIR instrument was purchased from Thermo Fisher Scientific (Model: Nicolet iS5, Britain). The samples were analysed using the ATR method in the region 4000 to 500 at a resolution of 4 cm⁻¹ and 16 scans. ¹H and ¹³C NMR Spectra of raw SCB and its derivatives (i.e., lignin and cellulose) were recorded using a Bruker spectrometer at 400.2124713 MHz. All the samples were dissolved in DMSO, and the acquisition time (AQ) and relaxation delay (D1) were set to 4.0894465 and 1 sec, respectively.

1.3 Quantitative Analysis

A gravimetric method was used to determine the percentage content of lignin and cellulose in raw SCB and pretreated SCB. Gravimetric analysis is a widely accepted, accurate technique for biomass composition studies. The percentage content was calculated from the difference between the initial and final weight using equation 1.

$$\text{Percentage Content} = \left(\frac{\text{Initial Mass} - \text{Final Mass}}{\text{Initial Mass}} \right) \times 100 \quad (1)$$

2 Results and discussion

2.1 Quantification analysis

The quantification of cellulose and lignin was done on raw SCB, SCB-Cellulose and SCB-Lignin as presented in Table 1. Raw SCB is mainly comprised of cellulose (97.4%) with a small amount of lignin (3.4%). These

values differ from the raw SCB content reported in the literature, which could be attributed to the source from which the SCB was collected [14]. After pretreatment, cellulose content increased (98.5%), and lignin content decreased. The content of Lignin recovered from the filtrate (i.e., black liquor) was 79.2%, with some cellulose impurities (20.9%). Moreover, the combination of H₂SO₄ and NaOH clearly demonstrated its effectiveness in fractionating both the cellulose and lignin. High removal of lignin and cellulose using the acidic and alkaline chemicals is similar to that reported in the literature [14, 18]. The selected pretreatment process was more effective than some reported methods (Table 2).

Table 1. Quantification of Cellulose and Lignin Biopolymers.

Samples	Cellulose (%)	Lignin (%)
raw SCB	97.4	3.9
SCB-Cellulose	98.5	2.1
SCB-Lignin	20.9	79.2

Table 2. Comparison of the biopolymer content of sugarcane bagasse after pretreatment with different chemicals.

Chemicals	Content %			Reference
	Cellulose	Hemicellulose	Lignin	
H ₂ O ₂	70.70	21	4.21	[14]
H ₂ SO ₄	55.51	5.57	26.48	[14]
NaOH	67.36	11.17	11.42	[14]
H ₂ SO ₄ , NaOH	65.03	10.95	8.12	[18]
H ₂ O ₂	53.85	22.02	7.99	[18]
NaOH	47.21	29.29	4.31	[18]
NaOH	35.7	18.1	14.0	[19]
NaOH	32.3	16.3	4.7	[19]
NaOH	32	15.7	1.8	[19]
Deep eutectic	64.74	15.93	15.58	[20]
H ₂ SO ₄	51.70	15.80	26.6	[20]
NaOH	73.88	13.17	9.09	[20]
H ₂ SO ₄ , NaOH	98.5	-	2.1	Present study

2.2 Characterisation of material

Different characterisation and analytical techniques were employed to study the physicochemical properties of the raw SCB and fractionated SCB-Cellulose and SCB-Lignin biopolymers. Figure 2 shows the Image of recovered SCB-Cellulose and SCB-Lignin.

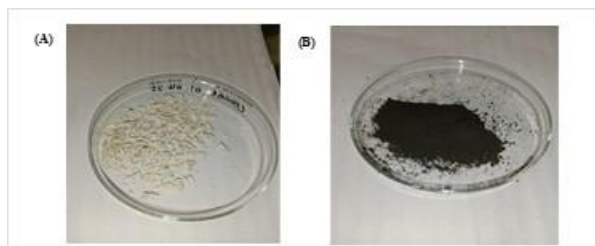


Fig. 2: Image of chemically extracted SCB-Cellulose (A) and recovered SCB-Lignin (B).

2.3 FTIR analysis

The FTIR spectra of raw SCB and its derivatives are presented in Figure 3. According to the results, raw SCB exhibits vibration absorption peaks at 338.23 and 2897.37 cm^{-1} , corresponding to the O-H and C-H stretching vibrations, which are correlated with the presence of cellulose, hemicellulose, and lignin molecules [21]. The vibration of O-H and C-H functional groups was also observed on extracted cellulose and lignin. However, the intensity of vibration peaks decreased, especially for SCB-Lignin. In contrast, the C-H was more visible on lignin and cellulose molecules. The absorption band observed at 1603.74 and 1541 cm^{-1} was attributed to the aromatic (C=O, C=C) structure bound to unsaturated functional groups [22]. These functional groups were significantly decreased on cellulose but were more visible on lignin. The high intensity of aromatic functional groups indicates that lignin was successfully extracted from the raw SCB. Furthermore, the absorption bands observed at 1160.08 and 1242.42 cm^{-1} could be correlated to the C-O-C groups. The absorption bands recorded at 833 cm^{-1} could be due to the C-H stretching of aromatic groups [22].

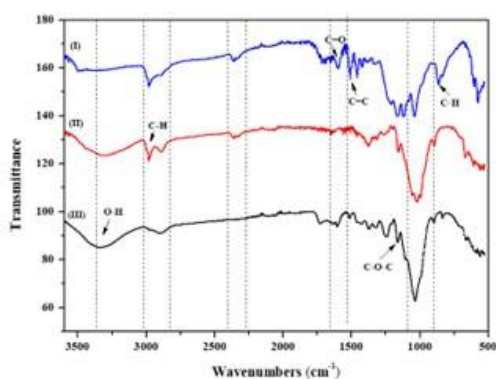


Fig. 3. FTIR spectra of recovered SCB-Lignin (I), extracted SCB-Cellulose (II), and raw SCB (III).

2.4 NMR analysis

The ^1H NMR spectra of raw SCB, SCB-Cellulose, and SCB-Lignin are presented in Figures 4 and 5. The signal peak at 2.5 ppm could be attributed to the DMSO- d_6 solvent [23]. ^1H - ^{13}C NMR detected both the

aliphatic sidechains ($\delta\text{C}/\delta\text{H}$ 50–70/0.65–5.32) and aromatic regions ($\delta\text{C}/\delta\text{H}$ 115–170/6–8.5) on lignin biopolymer [24]. The sidechains were also detected on raw SCB and cellulose biopolymer. Aromatic regions were detected in the raw SCB, which could be related to the presence of lignin, albeit with less intensity. However, the aromatic signals were not detected on cellulose, as expected, because the lignin had been removed. In addition, this shows that the extracted cellulose was highly pure. Furthermore, the sidechains consist of common linkages such as aryl ether (β -O-4, A), phenylcoumaran (β -5, C), and methoxy- OCH_3 [25]. The lignin spectrum shows a signal peak between 1.5 and 0.65 ppm, which could be correlated to the methylene groups of aliphatic chains [26]. The aromatic absorptions recorded at 7.52 and 6.27 can be attributed to guaiacyl and syringyl, respectively [27]. The signal peaks at 3.17 and 3.75 are correlated to methoxyl [27]. Moreover, the signals recorded at 4.27 ppm and between 4.87 and 5.32 ppm could be correlated to the H- β , H- α , and OH found in β -O-4', respectively [23]. Furthermore, these findings show that the combination of sulphuric acid and sodium hydroxide produces biopolymers with similar characteristics to those of other solvents, such as ionic liquids. However, future studies could focus on comparing different pretreatment methods, especially their impact on the final application of these biopolymers.

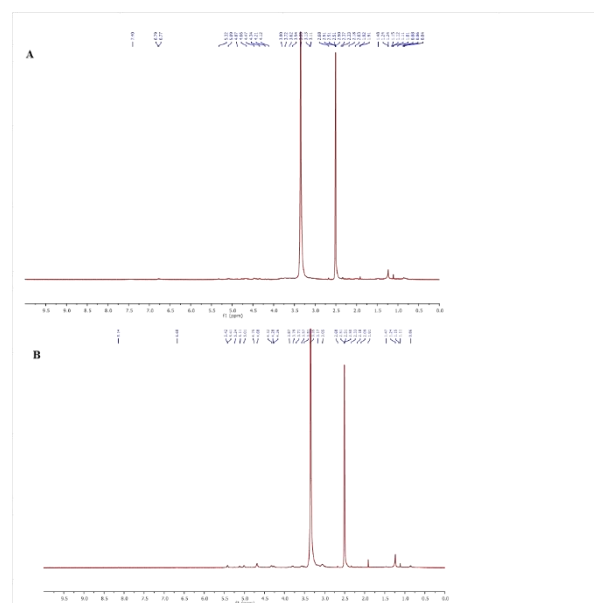


Fig. 4 ^1H NMR spectra of raw sugarcane bagasse (A) and extracted SCB-cellulose (B).

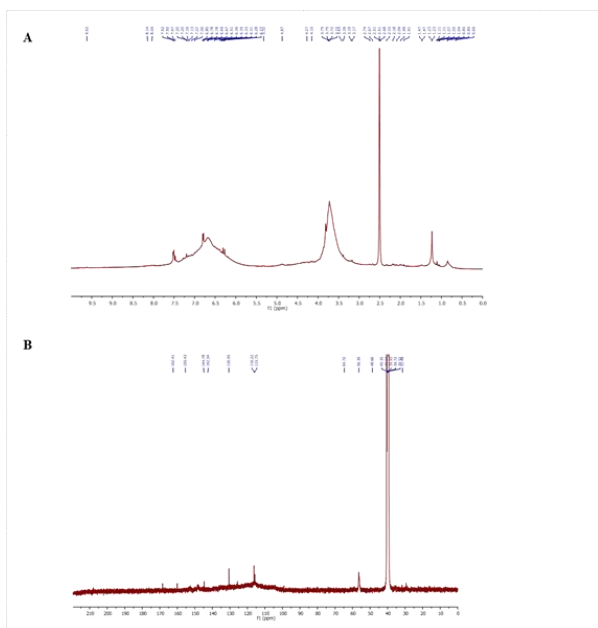


Fig. 5. ^1H NMR spectrum of recovered SCB-lignin (A) and ^{13}C NMR spectrum of SCB-lignin (B).

2.5 SEM analysis

Figure 6 shows the surface morphology of raw SCB, SCB-cellulose, and lignin recovered from raw SCB. According to Figure 6A, raw SCB shows a morphology that is smooth with packed fibers. The extracted cellulose shows a surface with porous and irregular structures, which can be attributed to the chemicals used during the pretreatment (Figure 6B) [26]. The surface morphology of SCB-Lignin exhibited irregular shapes and an uneven size distribution, consistent with previous studies (Figure 6C) [15].

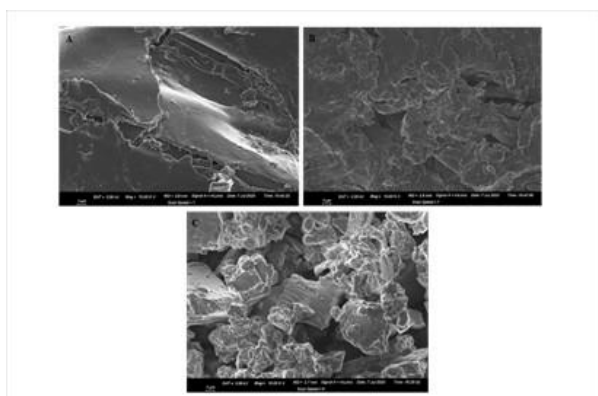


Fig. 6. SEM Image of raw SCB ; magnification: x10 000 (A), SCB-Cellulose ; magnification : x10 000 (B), and SCB-Lignin ; magnification : x10 000 (C).

3 Conclusion and recommendations

The cellulose and lignin biopolymers were successfully fractionated from raw sugarcane bagasse using diluted sulphuric acid and sodium hydroxide. The content of extracted SCB-Cellulose and recovered SCB-Lignin

was 98.5% and 79.2%, respectively. According to techniques such as FTIR, NMR, and SEM, the physicochemical properties of the biopolymers were also similar to those reported using other pretreatment methods, such as ionic liquids. Sulphuric acid and sodium hydroxide are not environmentally friendly compared to other solvents, but these chemicals are relatively inexpensive. Therefore, after the pretreatment, appropriate disposal of acidic or alkaline wastewater is recommended. Moreover, this study has demonstrated that the recovery of two or more biopolymers from raw sugarcane bagasse is possible, and this practice will minimise the waste generated in the environment. Future studies are encouraged to focus on the application of SCB-Cellulose and SCB-Lignin. Utilising both of these biopolymers will minimise the wastewater disposed of by biorefinery industries into the environment. These biopolymers can be utilised to produce valuable products, including biofuels, biochemicals, bioplastics, and biosorbents. In addition, future works are encouraged to investigate the impact of acid and alkaline methods on the final products produced from these biopolymers.

CRedit authorship contribution statement

Alusani Manyatshe: Conceptualisation, Writing- Original draft preparation. **Linda Lunga Sibali:** Conceptualisation, Supervision, Writing- Reviewing and Editing. **Vhahangwele Masindi:** Conceptualisation, Supervision, Writing- Reviewing and Editing.

Declaration of Competing interest

The authors declares that they have no known conflict of interest.

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