
PRELIMINARY SCREENING AND CHARACTERIZATION OF α -CELLULOSE EXTRACTED FROM COTTON STALK, SUGARCANE BAGASSE, AND SAWDUST USING ALKALINE PRETREATMENT

Hassana Gwandi Audu¹, • Prof. Ajayi Olusegun Ayoola^{1,*}, M.t Isa¹

¹Ahmadu Bello University Zaria

Email: euphorosagrocorpgreentech@gmail.com, segeaj@gmail.com*, mtisaz@yahoo.com

*Corresponding author: • Prof. Ajayi Olusegun Ayoola

DOI: <https://doi.org/10.67203/sp.ICESID.2026>

ABSTRACT

This study investigated the preliminary screening and characterization of α -cellulose from cotton stalk (CS), sugarcane bagasse (SCB), and sawdust (SD) to establish practical process parameters for subsequent optimization. A uniform particle size of 125 μm and solid-to-liquid ratio (1:10 w/v) were applied. CS was treated with 7.5–27.5 g NaOH/100 mL at 90°C for 1.5 h, while SCB and SD Method 1 were treated with 10–30 g NaOH/100 mL at 95°C for 1.5 h. SD Method 2 incorporated an initial 7 mL HNO_3 pretreatment at 95°C for 1.41 h prior to alkali treatment. Recovered fractions were evaluated by α -cellulose yield, purity, Van Soest fiber analysis, FTIR, XRD, and SEM–EDX. CS2 produced 30% yield and 84.06% purity; SCB2 produced 20% yield and 83.82% purity; SD3 produced 80% yield and 85.71% purity; and SDA3 produced 20% yield and 90.14% purity. FTIR confirmed substantial solubilization of non-cellulosic components. XRD analysis demonstrated shifts in Crystallinity Index (CrI) from raw to treated states: CS RAW (61.5%) to CS2 (61.4%), SCB RAW (55.7%) to SCB2 (59.6%), SD RAW (51.3%) to SD3 (66.1%), and reaching 75.2% for dual-pretreated SDA3. SEM–EDX revealed microfiber exposure, matrix disruption, and mineral leaching. Direct-alkali SD3 was selected as the optimal practical condition for sawdust due to high mass recovery (80%), process simplicity, avoiding acid consumption and multi-stage washing, where the +4.43% purity increase in SDA3 did not justify the 75% loss in recovered yield and added reagent cost. The selected conditions provide experimental starting points for subsequent DOE–CCD multi-pulping optimization; DOE–CCD and ANN/FFNN modelling are not claimed as completed in this preliminary study.

Keywords: α -cellulose; cotton stalk; sugarcane bagasse; sawdust; alkaline pretreatment; FTIR; XRD; SEM–EDX

1. INTRODUCTION

Lignocellulosic agricultural and wood residues are renewable resources containing cellulose, hemicellulose and lignin and offer important feedstocks for value-added cellulose-based materials (Riseh *et al.*, 2023). However, the close association of cellulose with hemicellulose and lignin makes direct recovery difficult. Chemical pretreatment, particularly alkaline treatment, can disrupt lignin–carbohydrate associations, promote fiber swelling and facilitate removal of non-cellulosic components, thereby increasing cellulose accessibility (Abolore *et al.*, 2023; Hassana *et al.*, 2021).

Cotton stalk, sugarcane bagasse and sawdust have different lignocellulosic structures and therefore require biomass-specific pretreatment conditions (Darmenbayeva *et al.*, 2026). Preliminary screening is consequently important for identifying suitable treatment conditions before subsequent process optimization. In this study, FTIR spectroscopy was used to evaluate changes in characteristic lignin, hemicellulose and cellulose functional groups through peak positions and transmittance responses. In this study, sodium hydroxide concentration was varied to screen suitable extraction conditions for the three biomasses, while an additional HNO_3 –NaOH pretreatment was examined for sawdust.

The aim of this work is to produce α -cellulose from cotton stalk, sugarcane bagasse and sawdust by varying the concentration of sodium hydroxide applied to the three biomasses, with an additional HNO_3 –NaOH route examined for sawdust only, in order to compare the two methods and identify the optimum preliminary conditions. The specific objectives were to evaluate the effect of NaOH

concentration on α -cellulose extraction; determine cellulose, hemicellulose and lignin fractions using the Van Soest method; characterize the extracted α -cellulose using FTIR, XRD and SEM–EDX; compare the alkaline and HNO_3 –NaOH routes for sawdust; and identify the preliminary optimum treatment conditions as a basis for subsequent RSM–CCD optimization.

2 METHODOLOGY

2.1 Biomass Preparation and Pretreatment

Cotton stalks were collected from a farm in Funtua - Katsina State, sugarcane bagasse from Samaru- Zaria and mixed sawdust from a local timber market Sabon Gari market Zaria. Cotton stalk and sugarcane bagasse were air dried for 2 months, while mixed sawdust was dried for a week to reduce the moisture content to 5%. The dried materials were pulverized using a Retsch mechanical grinder and ball mill to further reduce the particle sizes. An Endecotts test sieve (120 mesh; 125 μm apertures) was used to sieve the pulverized biomass to a uniform particle size of 125 μm . Ten grams (w/w) of biomass was used for each preliminary treatment. A constant solid-to-liquid ratio of 1:10 (w/v) was maintained throughout the pretreatment experiments, using 10 g of biomass and 100 mL of distilled water for each treatment, while varying the NaOH loading. For cotton stalk, CS1–CS5 were treated with 7.5, 12.5, 17.5, 22.5, and 27.5 g NaOH, respectively, at 90°C for 1.5 h in a water bath. Following pretreatment, the samples were washed repeatedly with distilled water until pH 5–7, filtered, oven-dried at 105°C for 3 h, and weighed. Similarly, sugarcane bagasse (SCB1–SCB5) and sawdust Method 1 (SD1–SD5) were treated with 10, 15, 20, 25, and 30 g NaOH, respectively, at 95°C for 1.5 h in a water bath, followed by washing to pH 5–7, filtration, oven drying and weighing. For sawdust Method 2 (SDA1–SDA5), the samples were initially treated with 7 mL HNO_3 diluted in 100 mL distilled water at 95°C for 1.41 h in a water bath. The samples were then washed to pH 5–7 and subsequently treated with 10, 15, 20, 25, and 30 g NaOH, respectively, at 95°C for 1.5 h in a water bath. The treated samples were then washed, filtered, oven-dried and weighed. The pretreatment conditions are summarized in Table 1.

Table 1: Preliminary pretreatment conditions used for biomass screening.

Biomass/method	Samples	NaOH (g/100 mL)	Temp. (°C)	Time (h)	Additional step
Cotton stalk	CS1–CS5	7.5–27.5	90	1.5	None
Sugarcane bagasse	SCB1– SCB5	10–30	95	1.5	None
Sawdust Method 1	SD1–SD5	10–30	95	1.5	None
Sawdust Method 2	SDA1– SDA5	10–30	95	1.5	7 mL HNO_3 + 100 mL H_2O ; 95°C; 1.41 h

2.2 Yield, Purity and Van Soest Fibre Analysis

Percentage α -cellulose yield was determined from recovered dry mass relative to the initial biomass mass. Purity was assessed using Van Soest detergent-fibre measurements through neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and ash add (Bartos et al., 2020). Hemicellulose was calculated from NDF (%) minus ADF (%), while cellulose and lignin were obtained from the corresponding fibre fractions. For NDF, 1.0 g of sample was placed in a round-bottom flask, followed by 100 mL neutral detergent solution and 0.5 g sodium sulphite, and refluxed at 100°C for 1 h. The residue was filtered, washed with hot distilled water to pH 5–7, rinsed with 20 mL acetone twice, oven-dried at 105°C for 1 h, and weighed in grams and recorded as NDF residue. For ADF, the dried NDF residue was transferred to the reflux flask, mixed with 100 mL acid detergent solution, and refluxed at 100°C for 1 h. The residue was filtered, washed with hot distilled water to pH 5–7, rinsed with 20 mL acetone twice, dried at 105°C for 1 h, and weighed (g) as ADF residue. For ADL and ash determination, the dried ADF residue was treated with 72% (v/v) H_2SO_4 for 3 h at room temperature with occasional stirring to solubilize cellulose. The remaining acid-insoluble residue was filtered, washed with hot distilled water until acid-free, dried to constant weight, and recorded in

grams as ADL residue. This residue was subsequently ashed in a muffle furnace at 550°C for 3 h and cooled in a desiccator, and the residual inorganic ash weight was recorded ash in grams. The equations for the Van Soest method is reported by Hassana *et al.*, 2021.

$$\text{Percentage Yield (\%)} = \frac{\text{mass of dried } \alpha\text{-cellulose}}{\text{mass of raw biomass}} \times 100 \quad \dots \dots \dots 1$$

$$\text{NDF (\%)} = \frac{\text{NDF residue (g)}}{\text{Original sample (g)}} \times 100 \quad \dots \dots \dots 2$$

$$\text{ADF (\%)} = \frac{\text{ADF residue (g)}}{\text{Original sample (g)}} \times 100 \quad \dots \dots \dots 3$$

$$\text{ADL (\%)} = \frac{\text{ADL residue (g)} - \text{Ash residue (g)}}{\text{Original sample (g)}} \times 100 \quad \dots \dots \dots 5$$

$$\text{Hemicellulose (\%)} = \text{NDF} - \text{ADF} \quad \dots \dots \dots 6$$

$$\text{Cellulose (\%)} = \text{ADF} - \text{ADL} \quad \dots \dots \dots 7$$

$$\text{Lignin (\%)} = \text{ADL} - \text{Ash} \quad \dots \dots \dots 8$$

$$\text{Percentage fraction of Purity (\%)} = \frac{\text{Cellulose}}{\text{cellulose} + \text{Hemicellulose} + \text{Lignin} + \text{Ash}} \times 100 \quad \dots \dots \dots 9$$

2.3 FTIR, XRD and SEM–EDX Characterization

FTIR was performed using a FTIR - CARY 630 FOURIER TRANSFORM INFRARED. The spectra were recorded over 4000–650 cm⁻¹ at a resolution of 4 cm⁻¹ resolution, 32 sample scans, 16 background scans, with Happ-Genzel apodization. Dried biomass samples were prepared as KBr pellets prior to analysis. The spectra were recorded in percentage transmittance (%T), and the characteristic absorption bands were identified from the resulting spectra (Ningthoujam *et al.*, 2023).

XRD analysis was performed using a Rigaku MiniFlex X-ray diffractometer with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) at 40 kV and 15 mA. Oven-dried powdered samples were scanned continuously over 3–70° (2 θ), using a step size of 0.02° at a scan speed of 20° min⁻¹. Crystallinity index (CrI) was calculated using the Segal empirical method, while crystallite size was estimated using the Scherrer equation with K = 0.89 (Sharma *et al.*, 2023). Peak positions and FWHM were obtained from the Rigaku evaluation software for the structural calculations. The equations used for the determination of these parameters were reported by French *et al.*, (2020); Monshi *et al.*, (2022).

SEM–EDX analysis was performed using a Phenom scanning electron microscope coupled with an EDX system. Powdered biomass samples were mounted on sample stubs using double-adhesive carbon tape and examined at magnifications of 500×–150,000×. EDX analysis was conducted at 15 kV by selecting representative regions of the SEM images for elemental analysis. The elemental composition was reported in atomic and weight percentages.

3. RESULTS AND DISCUSSION

3.1 Van Soest Fibre Composition of Raw and Pretreated Biomass Samples

The Van Soest results provided quantitative evidence of changes in the fibre fractions following pretreatment. The raw materials contained 25%, 28% and 34% cellulose for CS RAW, SCB RAW and SD RAW, respectively, while the treated samples generally showed reduced hemicellulose and lignin and increased cellulose-rich fractions (Bartos *et al.*, 2020). CS2, SCB2 and SD3 contained 58%, 57% and 60% cellulose, respectively, supporting their selection as practical preliminary starting

conditions. SDA3 gave 64% cellulose and 4% lignin, showing the stronger compositional effect of the acid–alkali route as presented in Table 2.

Table 2: Van Soest Fiber Composition of Raw and Calculated Fraction Purity of the Pretreated Biomass

Sample	NDF %	ADF %	ADL %	Ash %	Hemicellulose %	Cellulose %	Lignin %
CS RAW	68	44	19	2	24	25	19
CS1	60	55	9	2	5	46	9
CS2	69	65	7	1	4	58	7
CS3	66	60	8	1	6	52	8
CS4	64	59	7	1	5	52	7
CS5	61	56	6	2	5	50	6
SCB RAW	80	56	24	1	29	28	23
SCB1	60	55	10	1	5	45	10
SCB2	68	65	8	2	3	57	8
SCB3	63	57	7	1	6	50	7
SCB4	65	59	6	1	6	53	6
SCB5	61	55	5	3	6	50	5
SD RAW	80	56	22	2	24	34	22
SD1	61	55	10	2	5	45	10
SD2	66	61	8	2	5	53	8
SD3	70	66	6	1	4	60	6
SD4	68	62	5	2	6	57	5
SD5	65	59	4	3	6	55	4
SDA1	60	56	8	1	4	48	8
SDA2	67	63	6	1	4	57	6
SDA3	71	68	4	1	3	64	4
SDA4	69	64	3	1	5	61	3
SDA5	66	60	3	1	6	57	3

3.2 Percentage Yield and Purity of Alpha Cellulose of CS, SCB, SD, and SDA

Alpha cellulose was extracted from cotton stalk (CS), sugarcane bagasse (SCB), and sawdust under two distinct processing regimes—Method 1 (SD) and Method 2 (SD-A)—to evaluate trade-offs between mass yield and chemical purity.

CS yields ranged from 29% to 30% (2.9–3.0 g) with purities between 76.67% and 84.06%, where CS2 achieved peak purity (84.06% purity, 30% yield) and CS4 (81.25% purity, 29% yield). SCB exhibited lower yields of 15–20% (1.5–2.0 g) while maintaining strong purity levels, led by SCB2 at 83.82% purity (20% yield). Method 1 treated sawdust (SD) provided the highest mass recoveries overall at 50–80% (SD4: 80% yield, 83.82% purity; SD3: 60% yield, 85.71% purity). Method 2 treated sawdust (SD-A) experienced reduced yields (20–30%) due to intensified delignification, but delivered the highest polymer purity, peaking at 90.14% for SD3A (20% yield).

Overall, while Method 2 (SD3A) achieved the absolute highest purity (>90%) across all feedstocks, peak-purity representatives—CS2 (84.06%), SCB2 (83.82%), and Method 1 SD3 (85.71%)—highlight key refinement thresholds, with Method 1 SD3 providing the optimal balance between throughput mass recovery (60%) and high chemical purity. The summary of the results is presented in Table 3.

Table 3: Van Soest Fibre Composition and Calculated Cellulose Fraction Purity

SAMPLE ID	Initial wgt (g) Raw sample	Final wgt dried α -cellulose (g)	% yield α - cellulose	% purity α - cellulose
CS1	10	3	30	76.66666667
CS2	10	3	30	84.05797101
CS3	10	3	30	78.78787879
CS4	10	2.9	29	81.25
CS5	10	3	30	81.96721311
SCB1	10	2	20	75
SCB2	10	2	20	83.82352941
SCB3	10	1.5	15	79.36507937
SCB4	10	2	20	81.53846154
SCB5	10	2	20	81.96721311
SD1	10	5	50	75
SD2	10	6	60	80.3030303
SD3	10	8	80	85.71428571
SD4	10	6	60	83.82352941
SD5	10	5	50	84.61538462
SD1A	10	2	20	80
SD2A	10	2	20	85.07462687
SD3A	10	3	20	90.14084507
SD4A	10	2	30	88.4057971
SD5A	10	2	20	86.36363636

3.3 FTIR Characterization

FTIR confirmed substantial chemical modification. CS2 showed disappearance of the carbonyl feature near 1720 cm^{-1} and reduction of the lignin-associated 1510 cm^{-1} region, with a clearer cellulose fingerprint. SCB2 similarly showed disappearance of the 1720 cm^{-1} feature and reduction of lignin-associated signals. In sawdust, Method 1 retained lignin aromatic skeletal vibrations, whereas SDA3 showed the strongest delignification response (Li *et al.*, 2023) and strong cellulose C–O stretching around $1019\text{--}1025\text{ cm}^{-1}$. This is presented in Figure 1a-c.

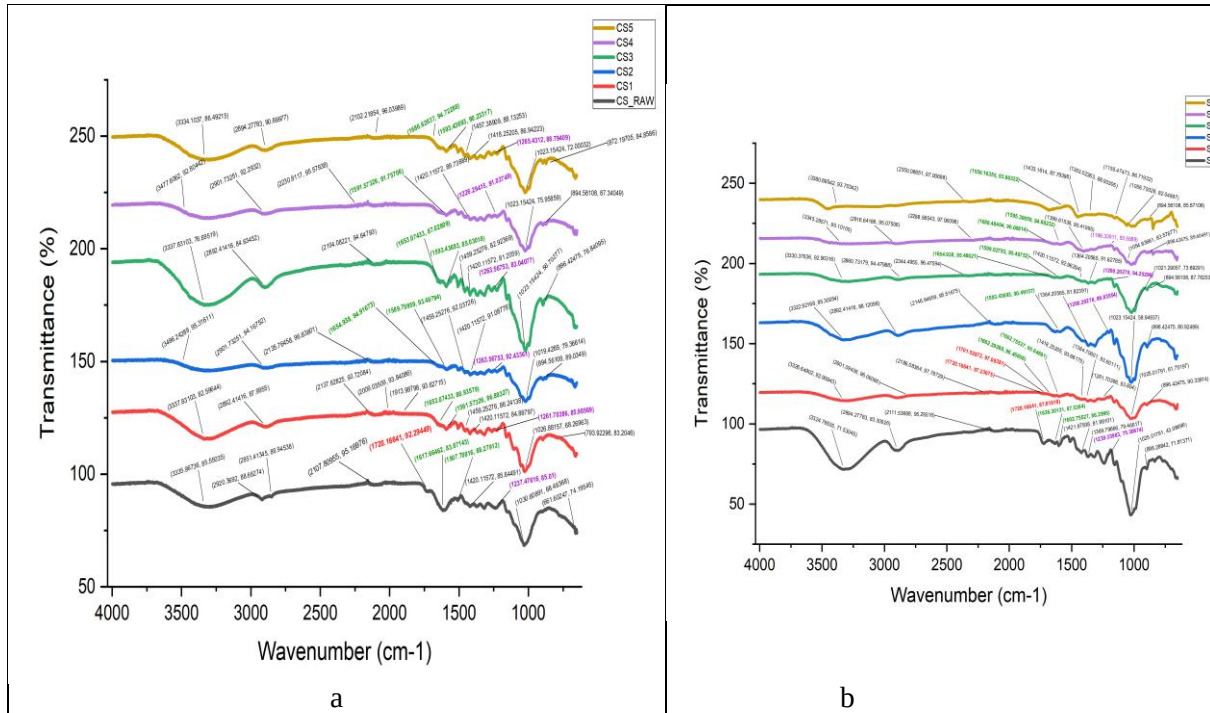


Figure 1. (a) FTIR spectra of untreated and treated cotton stalk (CS RAW and CS1–CS5), FTIR spectra of untreated and treated sugarcane bagasse (SCB RAW and SCB1–SCB5).

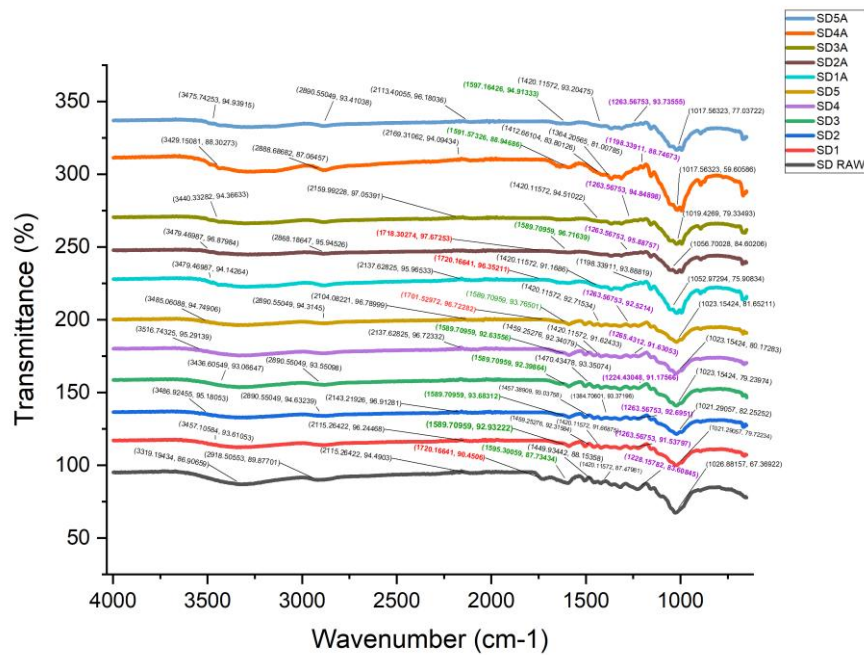


Figure 1c. FTIR spectra of untreated sawdust, Method 1 (SD1–SD5) and Method 2 (SDA1–SDA5).

The FTIR evidence supports the Fiber-fraction results. SDA3 showed the strongest evidence of delignification, but its higher purity was achieved through an additional acid-treatment route. SD3 therefore remained important as the simpler direct-alkali condition for subsequent optimization, yet with reasonable purity and yield.

3.4 XRD Characterization

XRD showed biomass-dependent structural changes. CS2 had a crystallinity index of 61.4%, compared with 61.5% for CS RAW; SCB2 increased from 55.7% to 59.6%; SD3 increased from 51.3% to 66.1%; and SDA3 reached 75.2%. The treated samples also showed changes in peak positions and crystallite size (Myeni et al., 2026). Origin-based interpretation indicated Cellulose I and Cellulose II-associated features in selected treated samples; CS2, SCB2 and direct-alkali SD3 retained native Cellulose I characteristics while displaying localized Cellulose II-associated polymorph features, whereas dual-treated SDA3 exhibited mixed Cellulose I/II-associated features with higher structural ordering as shown in Figure 2a–c.

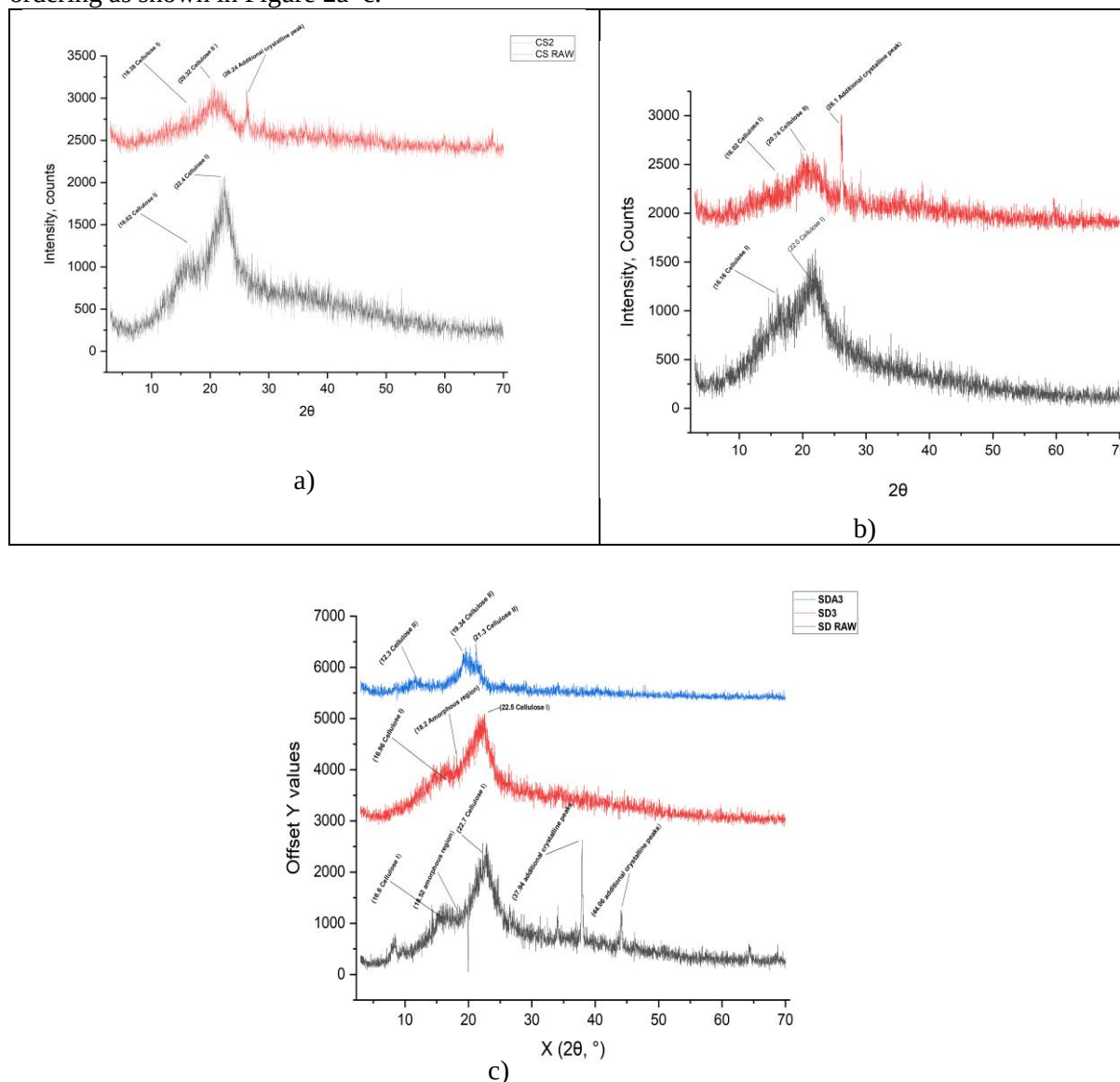


Figure 2: XRD patterns of untreated and treated a) cotton stalk (CS RAW and CS2), b) sugarcane bagasse (SCB RAW and SCB2) and c) untreated and treated sawdust (SD RAW, SD3 and SDA3).

3.5 SEM–EDX Characterization

SEM micrographs revealed clear morphological disruption following chemical treatment (Sunkar & Bhukya, 2022). CS2 displayed exposed fibrous bundles and irregular surfaces; SCB2 showed

disrupted, separated fibrous regions; and SD3 and SDA3 exhibited extensive, separated microfibers compared to compact raw matrices. EDX analysis confirmed accompanying elemental transformations.

For clarity, each entry in Table 4 reports the surface elemental composition first as atomic concentration (at.%) and then as weight percentage (wt.%) across the analyzed surface regions.

Regional carbon weight concentrations increased from 2.06 wt.% (6.07 at.%) in CS RAW to 11.39 wt.% (2.06 at.%) in CS2, and from 30.83 wt.% (54.85 at.%) in SCB RAW to 35.20 wt.% (30.83 at.%) in SCB2. For sawdust, regional carbon shifted from 8.22 wt.% (22.65 at.%) in SD RAW to 51.07 wt.% (70.51 at.%) in SD3, while SDA3 recorded 14.36 wt.% (36.42 at.%).

These shifts reflect the leaching of inorganic mineral extractives—notably potassium and calcium—and matrix disruption. However, because carbon is present across cellulose, hemicellulose, and lignin backbones, EDX was interpreted alongside FTIR and Van Soest analyses to confirm cellulose enrichment as presented in Table 4. Figure 3a-3c shows the SEM image of the raw samples and the selected optimum results.

Table 4: Summary of SEM–EDX observations for selected preliminary samples

Sample	SEM observation	EDX observation (at.% to wt.%)
CS RAW	Compact, aggregated fibre surface	C: 6.07 → 2.06 wt.%
SCB RAW	Compact, intact fibrous surface	C: 54.85 → 30.83 wt.%
SD RAW	Dense, compact wood-fibre surface	C: 22.65 → 8.22 wt.%
CS2	Exposed/separated fibrous bundles; irregular surface	C: 2.06 → 11.39 wt.%
SCB2	Disrupted and separated fibre regions	C: 30.83 → 35.20 wt.%
SD3	Exposed, separated and irregular fibres	C: 70.51 → 51.07 wt.%
SDA3	Strongly altered fibre surface	C: 36.42 → 14.36 wt.%

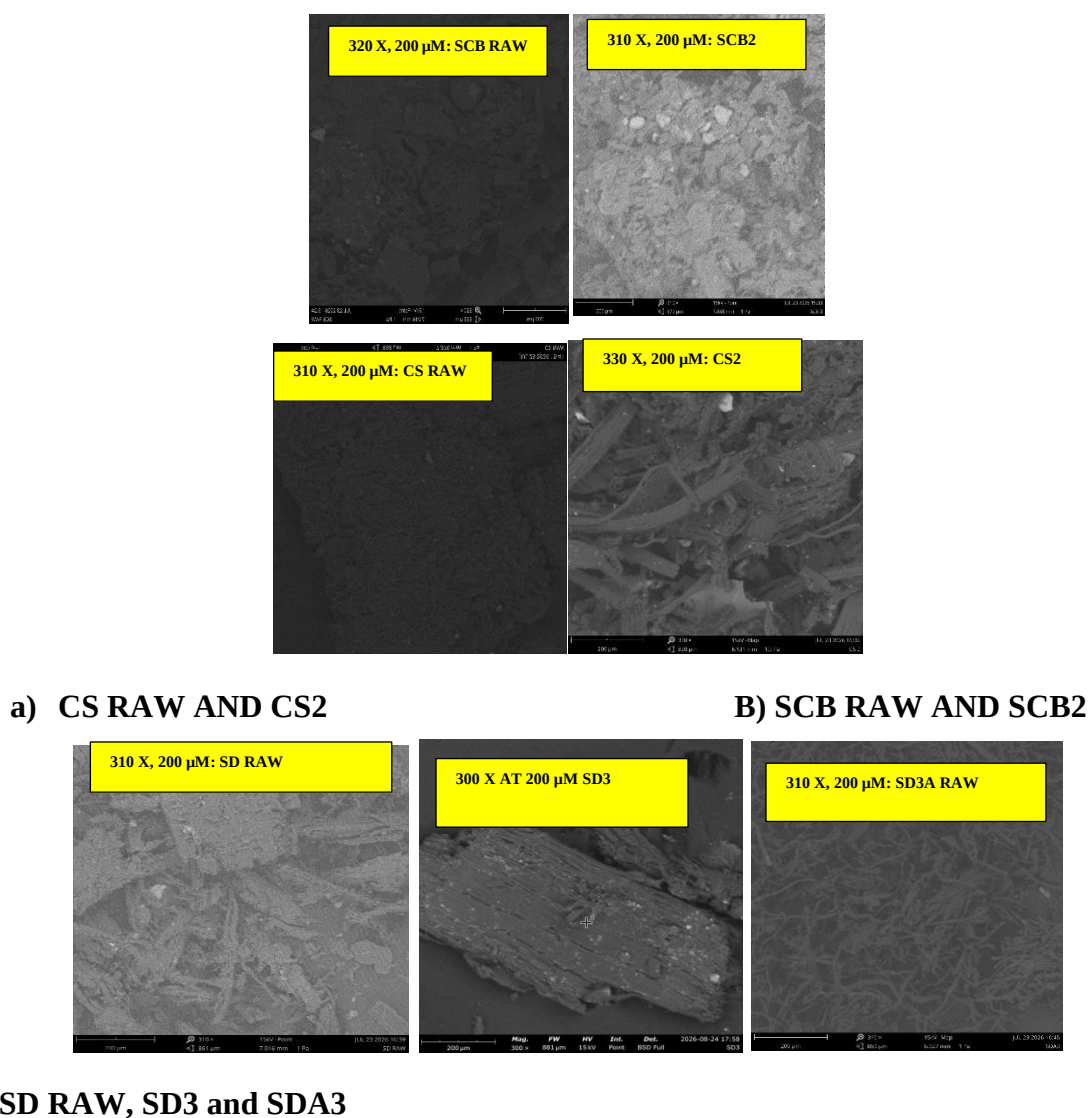


Figure 3: SEM Image of Raw Biomasses and Treated Samples a) CS RAW and CS2, b) SCB RAW and SCB2 and c) SD RAW, SD3 and SDA3

3.6 Selection of Preliminary Conditions

The preliminary selection was based on the combined consideration of α -cellulose yield, purity, Van Soest fibre composition, FTIR, XRD, and SEM–EDX rather than yield alone (Darmenbayeva et al., 2026). Among the evaluated feedstocks, CS2 (12.5 g NaOH, 90°C, 1.5 h) produced 30% yield and 84.06% purity, while SCB2 (15 g NaOH, 95°C, 1.5 h) produced 20% yield and 83.82% purity. For sawdust, Method 1 SD3 (20 g NaOH, 95°C, 1.5 h) produced 80% yield and 85.71% purity, whereas Method 2 SDA3 produced 20% yield and 90.14% purity. SD3 provides a yield that is 4 times higher than SDA3, representing a 75% drop in yield if switching to SDA3, or a 120% relative difference between the two data points. From an operational perspective, achieving the marginal 4.43% increase in purity via the SDA3 route incurs substantial resource overhead: it requires an additional acid-digestion stage using 3.5 (v/v) HNO₃, increases thermal energy consumption due to extended boiling cycles, extends total processing time by approximately 30-40%, and consumes significantly more deionized water for repetitive washing to reach neutral pH (7.0). This significant yield advantage, combined with the additional overhead costs in energy, time, and deionized water required by

alternative processes, justifies the selection of SD3 alongside CS2 and SCB2 for subsequent RSM–DOE–CCD multi-pulping optimization and predictive modelling (Rubia et al., 2024).

CONCLUSION

The present preliminary work established optimal processing regimes for α -cellulose extraction from cotton stalk, sugarcane bagasse, and sawdust feedstocks (Magalhães et al., 2023). CS2, SCB2, and SD3 were selected as practical starting conditions, producing 30%/84.06%, 20%/83.82%, and 80%/85.71% yield/purity, respectively. SDA3 produced a higher purity of 90.14% at 20% yield and showed stronger delignification and crystallinity, but required the additional acid-treatment route. SD3 was therefore preferred for the subsequent optimization because it provided a practical balance between yield, purity, structural quality, process simplicity, and reduced chemical requirements. FTIR, XRD, and SEM–EDX complemented the Van Soest analysis by demonstrating chemical, structural, morphological, and surface-elemental changes.

This study demonstrates how green chemical engineering frameworks can transform abundant local agricultural residues into functional bio-based precursors. Beyond process optimization, establishing these standardized extraction methodologies advances indigenous scientific knowledge and strengthens laboratory training and curriculum development in sustainable materials research across Nigerian universities. Furthermore, the purified α -cellulose produced serves as a critical structural feedstock for high-value applications across Nigeria's pharmaceutical sector (e.g., tableting excipients and binders) and the food and beverage industries (e.g., dietary fiber additives, thickeners, and active packaging materials). The selected preliminary conditions provide the experimental basis for the subsequent RSM–DOE–CCD multi-pulping optimization and later ANN/FFNN prediction stage.

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