Extraction and Characterization of Cellulose from Plant Biomass

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Abstract:
The growing demand for cellulose-based products has raised concerns about deforestation, prompting the search for sustainable alternatives. This study explores the extraction and characterization of cellulose from sugarcane bagasse and rice husk, both of which serve as abundant agricultural residues. The results demonstrated that sugarcane bagasse and rice husk yielded high-purity cellulose, highlighting their potential as eco-friendly alternatives to wood-derived cellulose. The extracted cellulose is suitable for paper production and other applications, including pharmaceuticals, food additives, and biodegradable packaging. Additionally, functionalized cellulose composites embedded with silver nanoparticles offer potential for antimicrobial applications in the medical and packaging industries. A pilot-scale process for the production of paper from the extracted cellulose was carried out, with the paper subjected to assessments of strength, flexibility, and utility (wettability, ink retention etc.). This research highlights the importance of utilizing agricultural residues in cellulose production, thereby contributing to environmental sustainability and promoting circular economy practices.

Keywords: Agricultural waste, delignification, acid hydrolysis, bleaching, Maule's test, Gram's iodine staining, paper production.

INTRODUCTION:

Cellulose ($(C_6H_{10}O_5)_n$) is a linear polysaccharide of β(1→4)-linked D-glucose units and a key structural component of plant cell walls. Hydrogen bonding between cellulose chains forms microfibrils, which give cellulose its high tensile strength. It's primarily used in paper production, with additional applications in textiles, biofuels, and biodegradable polymers.[1] Paper is made by suspending cellulose fibres in water, forming a sheet through draining, pressing, and drying. Sugarcane bagasse is the fibre residue that is left behind when juice is extracted from the sugarcane stalks in the production of sugar. Being rich in cellulose and low in silica content, bagasse is a suitable raw material for paper production[1][2], particularly in nations with limited forest resources. Its renewability at a quick pace, biodegradability, and low price also render it a good choice in the production of biofuels, compostable packaging, and building materials. Rice husk is the hard, protective outer shell separated from rice grains during the milling process. While it contains a fair amount of cellulose, its exceptionally high silica content (15–20%) makes it less suitable for traditional papermaking, as it causes equipment wear and leads to lower-quality paper due to its short fibre length.[3]

MATERIALS AND METHODS:

1) **Sample collection:**

Two types of plant-based agricultural waste were selected for this study

a. **Sugarcane (Saccharum officinarum) bagasse** was obtained from a local juice vendor in Santacruz, Mumbai.
b. **Rice (Oryza sativa) husk** was obtained from a rice mill in Dahanu.

The collected samples were transported to the laboratory, processed the following day, and stored at room temperature under appropriate conditions until further use.

2) **Sample Processing**

The pretreatment process involved washing rice husk and sugarcane bagasse (SCB) with water. The materials were then dried in a hot air oven, ground into smaller particles using a grinder, and sieved to obtain a uniform particle size.

3) **Extraction of cellulose:**

a. **Delignification (alkali treatment)**

Delignification removes lignin and partially dissolves hemicellulose, releasing fibrous cellulose. Rice husk and sugarcane bagasse (50–100 g) were treated with 0.5–10% NaOH (500–1000 mL) and heated under pressurized and non-pressurized conditions. The residue was filtered and washed with distilled water until neutral pH. A colour change indicated the efficiency of lignin removal.[4][5]

b. **Acid Hydrolysis (Hemicellulose removal)**

Acid hydrolysis is used to remove hemicellulose while retaining cellulose by breaking down plant material using acid and water. In this process, residues of rice husk and sugarcane bagasse from the alkali treatment were treated with 2–10% sulfuric acid (500–1000 mL) and heated under both pressurized and non-pressurized conditions. The mixture was then filtered to collect the cellulose-rich solid, which was washed with water until a neutral pH was achieved to eliminate residual acid.[4][5]

c. **Bleaching**

Pulp bleaching is a key step in papermaking, aimed at improving pulp brightness and achieving high-quality cellulose with desired whiteness, purity, and strength. In this study, hydrogen peroxide[5] (H_2O_2) at concentrations of 2–10% (w/v) was used. Additional batches were tested with varying sulfuric acid (H_2SO_4) concentrations to examine the effects of acid hydrolysis on bleaching efficiency. After treatment, the mixture was filtered, washed, and dried to yield purified, pulpy cellulose.

4) **Characterization of cellulose:**

A. Staining Techniques

For the detection of cellulose **Gram's Iodine staining**

Cellulose, a carbohydrate, forms a blue-black complex with iodine or potassium iodide. Gram's iodine was used to rapidly detect cellulase-producing microbes by highlighting clear zones around colonies[6]. Treated fibres were then stained and observed under a 40X microscope to examine their fibrous structure.

For the detection of lignin **Weisner staining**

Lignin provides structural support in plants. In sugarcane bagasse (SCB), safranin staining was used to visualize lignin. Unlike the typical Wiesner (phloroglucinol-HCl) method, safranin selectively binds lignin, aiding in identification.[7][8]

Maule's staining The Maule test identifies lignin in plant materials through sequential chemical treatments. Potassium permanganate oxidizes lignin components, followed by exposure to acid and ammonia, forming a coloured complex. A red coloration post-ammonia treatment confirms lignin presence. This method evaluates lignin content after delignification, with colour intensity reflecting solubilization efficiency and success of removal, leaving behind only pure cellulose.[7]

B. **Spectroscopic Techniques**

Fourier Transform Infrared Spectroscopy - FTIR measures the absorption wavelengths of infrared light to obtain the spectrum of the sample. FTIR spectroscopy provides details about the presence or absence of specific functional groups and can give an even deeper insight into the fibre's structure. FT-IR spectroscopy has been extensively used in cellulose research, since it presents a relatively easy method of obtaining direct information on chemical changes that occur during various chemical treatments.[5]

5) Determination of the percentage yield of cellulose

$$\frac{\text{dry weight of extracted cellulose}}{\text{dry weight of biomass (sugarcane bagasse or rice husk)}} \text{ x } 100$$

The formula above was used to estimate the percent yield of cellulose from the biomass.

6) Formation of a silver nanoparticle-embedded cellulose composite

The silver nanoparticles were synthesised from silver nitrate and sodium citrate. The final solution was a characteristic pale grey - brown solution. Deposition of nanoparticles on the surface of a variety of materials is a subject of great interest due to their potential applications in electronic devices, sensing, catalysis and bio-medical sciences. Antibacterial properties of polymer-supported silver nanoparticles are the subject of extensive studies for their numerous applications. The in-situ reduction of silver nanoparticles on cellulose fibres was carried out using sodium borohydride as a reducing agent.[8] The reduced metal nanoparticles are embedded in the cellulose fibres.

7) Production of Paper from the extracted cellulose

A 1M solution of NaOH was prepared, and the extracted cellulose pulp from the biomass was soaked in the alkaline solution for 30 minutes. Homogenized starch was later added to the aged pulp, and the mixture was heated to form a viscous solution. A 1:1 mixture of dilute sulfuric acid with sodium sulphate was prepared and added. The mixture was poured onto a mesh sieve over an aluminium vessel to drain the water, and the moulded cellulose was dried.

8) Assessment of the physical properties of the paper

1. Visual inspection – The surface of the paper was visually inspected for smoothness and evenness, ensuring there were no flaws or imperfections.
2. Touch test – The texture and smoothness of the paper were assessed by running fingers across its surface; the high-quality paper should feel refined and smooth, not rough.
3. Water holding capacity – Water holding capacity is the amount of water that can be absorbed or retained by a substance
4. Opacity – Opacity is the measure of how much light can pass through a sheet of paper.
5. Tear test – The strength of the paper was assessed by gently tearing a piece; a high-quality paper should resist tearing easily and hence be more durable.

6. Ink bleed test – Ink absorption was tested by writing on the paper with a marker and observing if the ink bled through to the other side; minimal bleed indicates good quality.
7. Thickness Measurement – It is essential for evaluating the structural integrity of paper. It determines the uniformity and strength of the material, ensuring consistency in quality and performance.

RESULTS AND DISCUSSION:

1. Extraction of Cellulose

Optimal delignification was achieved using 2% NaOH at a 1:10 biomass-to-solution ratio. A stronger colour change (yellow to dark brown) under pressure indicated improved delignification. The acid hydrolysis process was performed using H_2SO_4 with the optimal result observed at 2% concentration, with a ratio of 1 part of delignified biomass to 10 parts of the acid. The treatment was performed with and without pressure conditions. The results showed that the process was most effective at 2% concentration, with the best outcome achieved under pressure conditions, where the mass was further reduced to a sludge-like consistency.

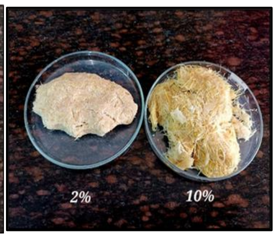



Fig.1 Delignified sugarcane bagasse. The removal of lignin has reduced the SCB to pulp. Fig. 2 (left) shows the SCB treated with 2% and 10% solutions of H_2SO_4 under pressure and later dried.

The bleaching process was conducted using H_2O_2 concentrations ranging from 2% to 10%, with optimal bleaching observed corresponding to the respective alkali-treated samples. The bleaching was carried out in a total volume of 100 mL solution of H_2O_2 in water. Additionally, a condition was tested where SCB underwent acid hydrolysis for one day without bleaching, and it was observed that SCB had lost the colour considerably, indicating that bleaching may not be a mandatory step under prolonged acid treatment conditions.

Fig. 3 The final mass of cellulose obtained after bleaching

2. Characterisation of cellulose

The staining tests provided clear insights into the structural changes in sugarcane bagasse during treatment. Before treatment, Wiesner staining using safranin confirmed the presence of lignin, as the red-to-pink coloration of mass structure was observed under light microscopy. After all the treatments, Gram iodine staining was performed, which revealed a bluish-black complex, confirming the presence of cellulose. The observation of distinct cellulose fibres under the microscope indicated a structural transformation from a mass-like form before treatment to a more **fibrous nature post-treatment, demonstrating the**

effectiveness of the delignification process.

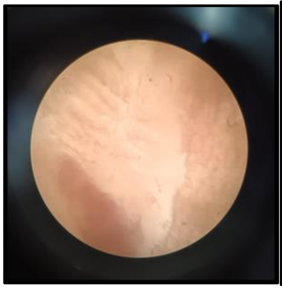
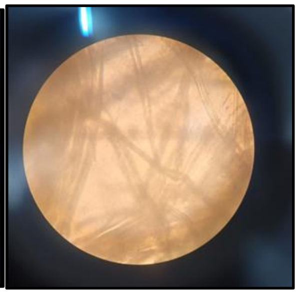



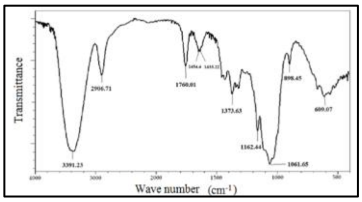
Fig. 5 Lignin visualised using Weisner Staining as dark regions representing a clustered mass of fibres. Fig. 6 The cellulose fibres visualised using Gram's iodine staining after treatment of the biomass. The strands appearing in a light, bluish grey colour are the cellulose fibres.

Maule's test was carried out to ascertain the presence of lignin in the sample after treatment and to assess the outcome of the delignification process. The filtrates obtained after delignification were tested, and the formation of a red colouration confirmed the presence of lignin.[7] The lignin content in rice husk was higher since the colour intensity and amount of filtrate undergoing colour change were higher than in the case of Sugarcane bagasse. The delignified sample was tested against the untreated samples, and the untreated samples displayed the presence of lignin by the means of a pink colouration being developed after the test procedure.


Fig. 7 (left) The Filtrate showing a red colouration after completion of the test. Fig. 8 (right) The treated and untreated sample subjected to Maule's test

3. Spectrometric analysis

Cellulose extracted from sugarcane bagasse was analysed using FTIR spectroscopy. All samples showed characteristic peaks for –OH stretching (3530–3050 cm^{-1}) and β-glycosidic linkages (900–1000 cm^{-1}). Peaks at 1515 cm^{-1} and 1732 cm^{-1}, linked to lignin and hemicellulose[5], were weak, indicating effective delignification and hydrolysis. Comparison showed that cellulose extracted with 2% NaOH and 2% H_2SO_4 yielded better results than using 10% concentrations



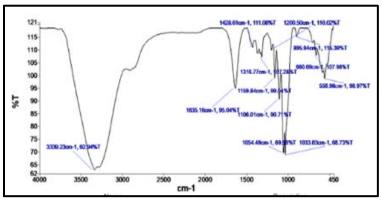


Fig. 9 FT–IR spectrum of standard cellulose. Fig. 10 FT–IR spectrum of cellulose extracted from SCB using 2% NaOH solution and 2% H_2SO_4

FTIR analysis of cellulose from rice husk showed weak peaks at 1515 cm^{-1} and 1732 cm^{-1}, indicating low lignin and hemicellulose content, and confirming effective delignification and hydrolysis. Samples treated with 2% NaOH and 2% H_2SO_4 showed deep, broad peaks, suggesting effective extraction. Comparison with sugarcane bagasse revealed that SCB yielded cellulose more efficiently than rice husk under similar treatment conditions.

4. Determination of the yield of cellulose

$$\text{percent yield of cellulose} = \frac{\text{dry weight of extracted cellulose}}{\text{dry weight of total biomass before treatment}}$$

SAMPLE TAKEN	CALCULATIONS	RESULT
20 grams of SCB	(10.042 / 20.000) x 100	50.21 %
50 grams of SCB	(22.543 / 50.000) x 100	45.09 %
100 grams of SCB	(48.076 / 100.000) x 100	48.08%
20 grams of rice husk	(8.942 / 20.000) x 100	44.71 %
50 grams of rice husk	(15.653 / 50.000) x 100	31.31%
100 grams of rice husk	(42.548 / 100.000) x 100	42.55%

Table 4: Percent yield of cellulose obtained from various amounts of SCB and rice husk.

5. Formation of cellulose–silver nanoparticle composite

The silver nanoparticles were synthesised using a diluted solution of silver nitrate and a solution of sodium citrate. In the borohydride reduction process, the colour of pre-soaked cellulose fibres (into metal salt solutions) changed immediately after dipping the cellulose fibres into sodium borohydride solution.[8]

Fig 11: Cellulose used as a matrix to create a composite with nanoparticles.

6. Assessment of Physical Properties of Paper for Application Suitability:

a. **Visual Inspection (Surface Quality & Flexibility):**

The paper had a smooth surface, indicating good fibre distribution and uniform density of fibre in almost all areas

b. Touch Test (Texture analysis):

Compared to standard writing paper, the texture felt coarse. This roughness suggests incomplete fibre breakdown or inadequate slurry refinement. A more viscous pulp mixture, could enhance uniformity and smoothness.

c. **Water Holding Capacity (Absorption Ability)**:
The paper displayed water good retention properties. This suggests a well-distributed cellulose network capable of holding moisture, which is essential for applications requiring absorbency, such as tissue or filter papers.


Fig. 12. The paper appeared to be bright, refined, and homogenized Fig. 13: The water-holding capacity of the paper was assessed.

d. **Opacity (Light transmission resistance)**
The paper exhibited moderate opacity. This suggests a dense fibre structure with minimal gaps.
e. **Tensile strength evaluation:**
The tensile strength of the paper was poor, as it exhibited weak tear resistance.

f. **Ink bleed test (ink absorption and porosity)**
When tested with a felt-tip marker, the ink did not bleed through the paper, suggesting moderate ink absorption. This indicates that the paper had sufficient porosity to retain ink without excessive spreading.

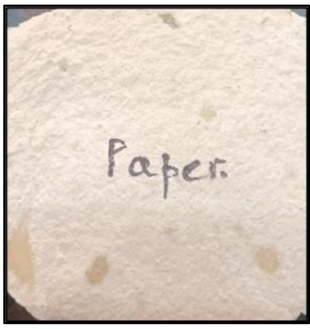


Fig. 14 Obverse side. Fig. 15 Reverse side

g. Thickness measurement (structural integrity)
The observed paper thickness was 1.5 mm, suggesting a well-formed sheet with good cellulose dispersion. The even spread of fibres during sheet formation likely contributed to this uniform thickness.

CONCLUSION:
Cellulose was extracted from sugarcane bagasse using a modified Kraft process with varying concentrations of NaOH and H_2SO_4.[4][5] The 2% concentration proved most effective, yielding brighter, more pulpy cellulose. Staining techniques confirmed efficient delignification: untreated samples showed dense red lignin masses (Wiesner stain), while treated ones displayed cellulose fibres (Gram's iodine). The Maule test further validated lignin removal, with pale pink coloration in treated samples indicating

reduced lignin.

FTIR analysis showed characteristic peaks at ~902 cm^{-1} (β-1,4 glycosidic linkages), confirming cellulose presence.[5] Peaks were broad and deep, indicating high cellulose content. Yield analysis proves sugarcane to be the more efficient source. Extracted cellulose was used to synthesize a composite with silver nanoparticles. Paper produced from this cellulose was bright and refined but lacked flexibility and tensile strength, likely due to low binder content. It showed high water absorption, low opacity, and good ink absorption without blotting.

FUTURE PROSPECTS:

The paper produced could be strengthened further by incorporating additives like binders, starch, or natural resins. This could also make the paper more flexible and durable, improving its suitability for various applications. Future studies can explore cellulose-based smart packaging, biomedical materials like wound dressings, and biodegradable films for wider industrial use. Environmental sustainability of agro-waste papermaking can be evaluated by comparing energy use, water consumption, and waste generation with conventional methods. Further investigation into the antimicrobial properties of the silver nanoparticle-cellulose composite is recommended. By fine-tuning the concentration of nanoparticles, the composite could be tailored for specific applications, such as surgical dressings or sutures, where antimicrobial properties are crucial.

REFERENCES:

1. Ajala, E.O., Ighalo, J.O., Ajala, M.A. et al. Sugarcane bagasse: a biomass sufficiently applied for improving global energy, environment and economic sustainability. Bioresour. Bioprocess. **8**, 87 (2021). https://doi.org/10.1186/s40643-021-00440-z
2. Rainey, Thomas J, and Noel Clark. "An Overview of Bagasse as a Resource for the Australian Paper Industry." International Sugar Journal, vol. 106, no. 1271, 1 Nov. 2004.
3. Nahla A. El-Wakil, Nesrin F. Kassem, Mohammad L. Hassan, Hydroxypropyl cellulose/rice straw oxidized cellulose nanocrystals nanocomposites and their use in paper coating, Industrial Crops and Products, Volume 93, 2016, Pages 186-192, ISSN 0926-6690,https://doi.org/10.1016/j.indcrop.2016.02.026.
4. Mishra, S., Prabhakar, B., Kharkar, P. S., & Pethe, A. M. (2022). Banana Peel Waste: An Emerging Cellulosic Material to Extract Nanocrystalline Cellulose. ACS Omega. https://doi.org/10.1021/acsomega.2c06571
5. Mekonnen, M. Extraction of Cellulose from Sugarcane Bagasse Optimization and Characterization. Advances in Materials Science and Engineering. https://doi.org/10.1155/2022/1712207
6. Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A. A rapid and easy method for the detection of microbial cellulases on agar plates using gram's iodine. Curr Microbiol. 2008;57(5):503-507. doi:10.1007/s00284-008-9276-8
7. Crocker, E. C. (2017). MAULE LIGNIN TEST ON PODOCARPUS WOOD. In Botanical Gazette (Vol. 951). http://www.journals.uchicago.edu/t-and-c
8. Yamashita, D., Kimura, S., Wada, M. et al. Improved Mäule color reaction provides more detailed information on syringyl lignin distribution in hardwood. J Wood Sci **62**, 131–137 (2016). https://doi.org/10.1007/s10086-016-1536-9
9. Ashraf, S., Saif-ur-Rehman, Sher, F., Khalid, Z. M., Mehmood, M., & Hussain, I. (2014). Synthesis

of cellulose-metal nanoparticle composites: Development and comparison of different protocols. Cellulose, 21(1), 395–405. https://doi.org/10.1007/s10570-013-0129-7

10. R Salve and Ray, Ravindra and Subhajit. "Pharmacy Journal | Pharmaceutical Journal | the Pharma Innovation Journal." Www.thepharmajournal.com, 2020.
11. Pratama, Jeesica Hermayanti, et al. "The Extraction of Cellulose Powder of Water Hyacinth (Eichhornia Crassipes) as Reinforcing Agents in Bioplastic." 3rd International Conference on condensed matter and Applied Physics (ICC-2019), vol. Volume 2219, no. Issue 1 5 May 2020, 2020, https://doi.org/10.1063/5.0003804. Accessed 5 Jan. 2021.
12. Mishra, Shweta, et al. "Banana Peel Waste: An Emerging Cellulosic Material to Extract Nanocrystalline Cellulose." ACS Omega, vol. 8, no. 1, 28 Dec. 2022, pp. 1140–1145.
13. Casanova, F.; Freixo, R.; Pereira, C.F.; Ribeiro, A.B.; Costa, E.M.; Pintado, M.E.; Ramos, Ó.L. Comparative Study of Green and Traditional Routes for Cellulose Extraction from a Sugarcane By-Product. Polymers 2023, 15, 1251