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SUSTAINABLE PROCESS OPTIMIZATION FOR CELLULOSE RECOVERY FROM RICE HUSK AGRO-WASTE

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ABSTRACT
Rice husk, rich in lignocellulosic components, is an abundant agricultural waste and a promising renewable source for cellulose recovery. In this study, three different chlorine-free extraction methodologies were evaluated for the recovery of cellulose from rice husk agro-waste with a focus on process sustainability, yield, purity and structural integrity. The first method was ultrasound-assisted mild alkali treatment and hydrogen peroxide bleaching; the second method was ethanol-mediated Soxhlet dewaxing, stronger alkali treatment, autoclave assistance and hydrogen peroxide bleaching; and the third method was thermal pretreatment, ethanol/benzene Soxhlet extraction, intensified alkali treatment, repeated autoclaving and peracetic acid bleaching. The extracted cellulose samples were characterized by quantitative estimation of cellulose by the Updegraff–Anthrone method, solubility, scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD). The cellulose recovery increased progressively from RHC1 to RHC3, and the highest cellulose content was found to be above 78 % with RHC3 showing the best purification efficiency. SEM analysis showed increased separation of fibres and exposed fibrillar morphology in RHC3 indicating removal of lignin, hemicellulose, waxes and other non-cellulosic impurities. FTIR analysis confirms the presence of characteristic groups of cellulose and the removal of hemicellulose and lignin. XRD analysis also indicated the preservation of the native crystalline structure of cellulose and RHC3 possessed a crystallinity index of 78.2%. Overall, the third strategy was optimized and found to be most effective to obtain crystalline cellulose of high purity from rice husk. The study supports the valorization of rice husk waste to value-added cellulose for possible applications in sustainable biopolymer composites, biomedical materials, and hydrogel-based systems.
KEYWORDS: Rice husk, Cellulose, Bioconversion, Sustainable materials, Waste valorization, Biopolymer.

1. INTRODUCTION
The uncontrolled human activities significantly contributed to environmental changes throughout the ages, in the forms of increased emissions of greenhouse gases, climate change, and global warming. Among the most pressing environmental concerns related to these anthropogenic activities, there is the accumulation of solid wastes related to industrial, agricultural, and medical sources. Agricultural residues constitute a major fraction of the global biomass waste as well as present a significant challenge in disposition. This type of waste is often burned or dumped in landfills, which causes secondary pollution and additional greenhouse gas emissions (Blasi et al.,2023, Nair et al.,2022). Therefore, sustainable utilization and use of agricultural residues have received significant focus over the last few years.
Agricultural residues have a high content of lignocellulosic biomass and represent a renewable, low-cost, and inexpensive feedstock to produce value-added biopolymers, especially cellulose and lignin. Rice husk, a major by-product of rice milling, is particularly abundant in rice-producing countries like India. It is also estimated that it has about 38-40% cellulose, 20-25% hemicellulose and 15-20% lignin, although its composition is affected by the rice genotype and environmental factors. Due to the richness of its cellulose and the availability worldwide, rice husk has become one of the promising raw materials in cellulose extraction and its subsequent use in fields like nanotechnology, water purification, and biopolymer which is sustainable (Malik et al.,2024).
The most abundant natural polymer on earth is cellulose, which is a linear polysaccharide, a polymer of D-glucose units, bonded by 1,4-glycosidic bonds. Its biocompatibility, biodegradability, and mechanical strength make it very important in a broad spectrum of biomedical, pharmaceutical and industrial applications. Nevertheless, recovery of cellulose in rice husk is not easy since cellulose is encircled with a multifaceted lignocellulosic web created by hemicellulose and lignin. These non-cellulosic compounds serve as physical cum chemical barriers, decreasing the availability of cellulose and significantly lowering the efficiency of hydrolysis and conversion. The lignocellulosic biomass even without treatment, will have low cellulose conversion rates which can be

significantly enhanced after the right pre-treatment (Rashid et al.,2021). Therefore, lignin and hemicellulose must be removed effectively to achieve the highest level of purified cellulose that can be used in high-tech applications. Structurally, hemicellulose is a heterogeneous, and branched polysaccharide which is made of different sugar monomers that are joined by β-1,3 and β-1,4 glycosidic bonds that make it more amorphous and susceptible to thermal and chemical degradation. On the contrary, cellulose is a repeating unit of cellobiose that create a highly crystalline structure supported by many intra and intermolecular hydrogen bonds, which imparts resistance to both swelling in water and enzyme degradation. Lignin, which is an amorphous and highly cross-linked phenylpropanoid polymer, further enhances the structural rigidity of the biomass and complicates cellulose extraction.

To overcome these challenges, various cellulose extraction techniques have been developed, among which are alkali treatment, bleaching, acid hydrolysis and solvent-based processes. Advanced enhanced methods of pretreatment including steam explosion processing and organosolv processing have shown improved delignification, and cellulose recovery. For instance, Steam explosion has been known to obtain cellulose extraction efficiencies approx. 70% (Serrano-Martinez et al.,2024), while organosolv treatment has been reported to remove up to 82 % lignin and hemicellulose with a crude cellulose purity of about 78% (Aggarwal et al.,2021). More recently, formic and peroxyformic acid treatment has been found to extract cellulose nanocrystals of rice husk with improved crystallinity (Vu et al.,2024). Despite this advancement, achieving high cellulose yield and purity remains challenging due to the inherent complexity of the rice husk matrix, which highlights the need for optimizing sustainable means of extraction.

Cellulose extraction typically carried out sequentially chemical and mechanical treatments to remove lignin, hemicellulose, and silica from rice husk. Lignin and silica dissolution using sodium hydroxide is usually done at higher temperatures to increase the access of cellulose and the crystallinity (Sharma et al.,2018). Further bleaching steps remove the remaining lignin and chromophoric impurities. Over the last few years, non-chlorine based bleaching reagents like hydrogen peroxide and peracetic acid have been gaining popularity because of their less harmful effects on the environment (Hincapie et al.,2024). Hydrolysis with acid is occasionally added to selectively cleave amorphous areas to produce microcrystalline cellulose that possesses superior functional properties (Ahmad et al.,2026).

The valorization of agricultural waste such as rice husk, aligns closely with the principles of sustainable development like resource efficiency, reduction of waste, and environmental conservation. The study of sustainable materials is shifting towards more topics on using agro-industrial waste to produce biodegradable and biocompatible materials as well as high value products (Chung et al.,2022). For example, rice husk-derived cellulose has been effectively converted to nanofibers using alkali treatment followed by peracetic acid bleaching which allows the separation of pectin, waxes, and other extractives (Gupta et al.,2020). In this study, we present a comparative analysis of three different cellulose extraction strategies from waste rice husk is presented. Each method varies with the stage of pretreatment, delignification degree and bleaching. The yield, purity, solubility, morphology and structural integrity of the extracted cellulose were analyzed methodically, by using, FTIR, SEM and XRD. The primary objective of this work is to identify an efficient, environmentally friendly, and scalable extraction pathway, which makes a contribution to the waste-to-wealth valorization of the agricultural biomass and its potential application in biomedical and sustainable material development (Pandey et al.,2026).

2. MATERIALS AND METHODS

2.1. Materials

Rice husk (SK SORTEX Pvt. Ltd., DURG, Chhattisgarh). Methanol 99.5 % (CODE-00195) from LOBA CHEMIE PVT. LTD. and Benzene Crystallizable 99% (CODE- 00038) from LOBA CHEMIE PVT. LTD., Sodium hydroxide pellets, Hi-LR™ (Catalog. No.- TC460) from HI-MEDIA, Acetic acid Glacial 99.5% (CODE- 00004) LOBA CHEMIE PVT. LTD., Hydrogen Peroxide Solution (CODE- 0183A) and Sulphuric acid 98% (LOBA CHEMIE PVT. LTD.), Millipore water purification system ICW 3000 is used to get the ultrapure water. The glassware utilized is purchased from BOROSIL.

2.2. Pre-treatment and Extraction Strategies:

Rice husk (RH), an agricultural by-product, was obtained from S.K. Industries rice mill in Chhattisgarh, India. A variety of rice hulls, or husks (RH), were employed in the rice mill. The cellulose extraction routes were designed to be chlorine free to avoid the use of chlorinated bleaching agents such as sodium chlorite, sodium hypochlorite and chlorine dioxide. Instead, peracetic acid and hydrogen peroxide were selected as environmentally preferred oxidants for bleaching and delignification. The three strategies were characterised according to their extraction mechanisms. The collected husk was thoroughly washed many times with distilled water to remove the dirt particles, followed by air drying for 2-3 days in the daylight and open atmosphere. The dried husk material was then pulverized to produce fine powders with a particle size range of 100 to 250 µm. The powdered sample was further dried for 12 to 24 hours at 60°C in a hot air oven. Further to this, three different extraction strategies were employed.

2.2.1. Approach 1 (Ultrasound-Assisted Alkali Extraction and bleaching)

For alkali treatment, RH powder (20 g) was dispersed in 1% (w/v) sodium hydroxide (NaOH) solution with a sample to solution ratio of 1:20 g/mL. The suspension was sonicated in an ultrasonic bath operating at a frequency

of 40 kHz for 15–20 min at 45–50 °C. The suspension was stirred intermittently during sonication to ensure uniform exposure of RH particles to ultrasonic waves. The mixture was heated on a magnetic hot plate at 45–50 °C for alkali pretreatment after ultrasonication with constant stirring using a magnetic bead. After treatment, the residue was separated from the reaction mixture by physical filtration using Whatman filter paper and washed several times with distilled water until the filtrate attained a pH of about neutral. In the bleaching stage, 10 g of the dried alkali treated material was dispersed in 250 ml of hydrogen peroxide solution. A 3% (v/v) H_2O_2 solution was used for bleaching, and the pH of the bleaching medium was adjusted to 10–11 by adding dilute NaOH. The suspension was stirred for 4 h at 60 °C with continuous stirring. The fibres were separated by vacuum filtration and washed thoroughly with distilled water till the filtrate was at pH 6–7 after bleaching. The purified cellulose fibres were dried at 35–45 °C for 12–24 h in an air circulation oven. Finally, the dried cellulose sample was collected and stored in a clean glass vial and labelled RHC1 (Johar et al., 2012, Sharma et al., 2018**,** Gabhane et al., 2014, Gavrila et al., 2024) (Figure 1).

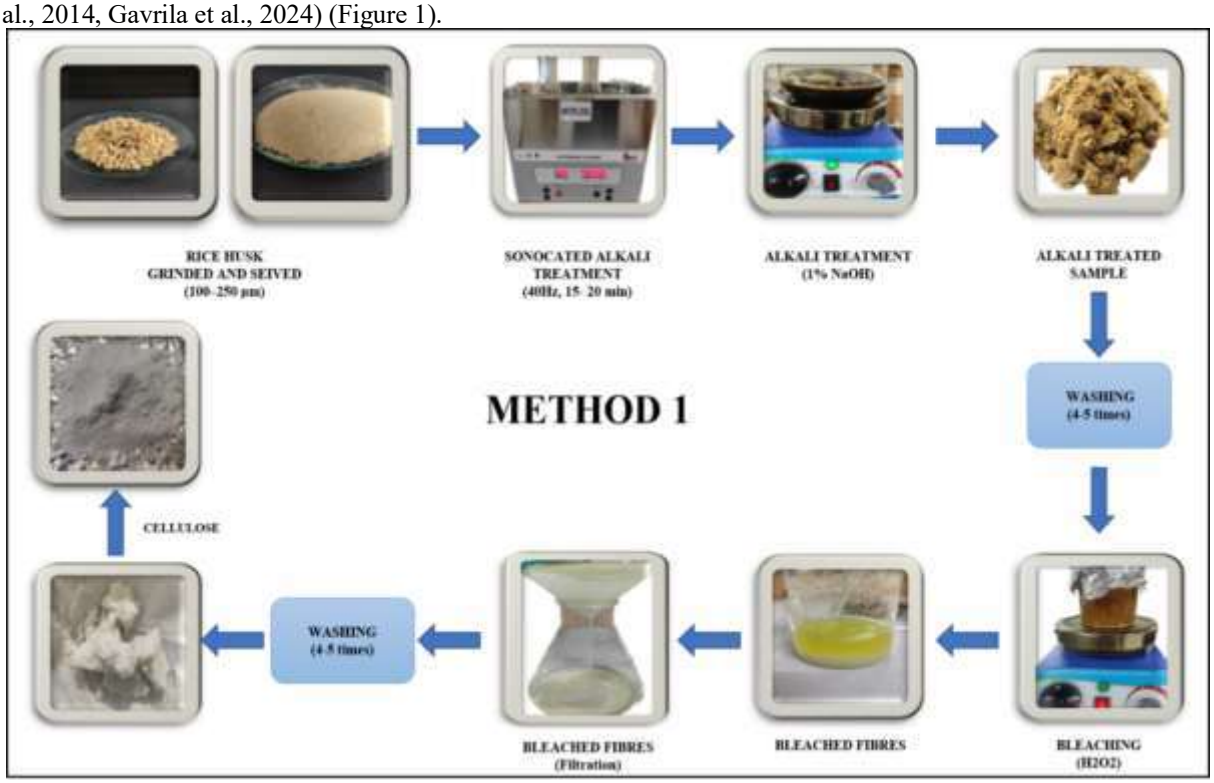

Figure1: Schematic representation for the extraction of cellulose from waste rice husk by Ultrasound-Assisted Alkali Extraction and bleaching method

2.2.2. Approach 2 (Ethanol-Mediated Solvent–Alkali–Autoclave-Assisted Extraction and bleaching)

The 10gm of dried Rice husk (RH) powder was subjected to dewaxing using Soxhlet extraction. In this step, 20 g of RH powder was extracted with 95% of ethanol for 4-6 h at 70-80 °C. The solvent treated RH powder was dewaxed, dried to remove residual ethanol and then alkali treated. The dried sample was dispersed in 3% (w/v) sodium hydroxide (NaOH) solution with a sample to solution ratio of 1:20 g/mL and was heated at 75 °C for 45 min under continuous magnetic stirring. The suspension was autoclaved at 121 °C and 15 psig following alkali treatment. After the alkali-autoclave treatment, the solid residue was separated from the filtrate by filtration and washed several times with distilled water until the pH of the filtrate was approximately neutral. The washed material was dried in a hot air oven at 60–70 °C for 24 h. The dried alkali treated material was then bleached with 250 mL of bleach solution. For a greener bleaching approach, a 3% solution of hydrogen peroxide at pH 10–11 adjusted with dilute NaOH may be used, heated at 60 °C for 4 h with continuous stirring. After bleaching, the fibres were separated by filtration and washed thoroughly with distilled water to pH 6-7. Finally, the purified cellulose fibres were dried in a hot air oven at 35–45 °C for 12–24 h. The dried cellulose sample was collected and stored in an airtight tubes/ container and labelled as RHC 2. (Pratama et al., 2020; Novia et al., 2022; Syamsidar et al., 2024) (Figure 2)

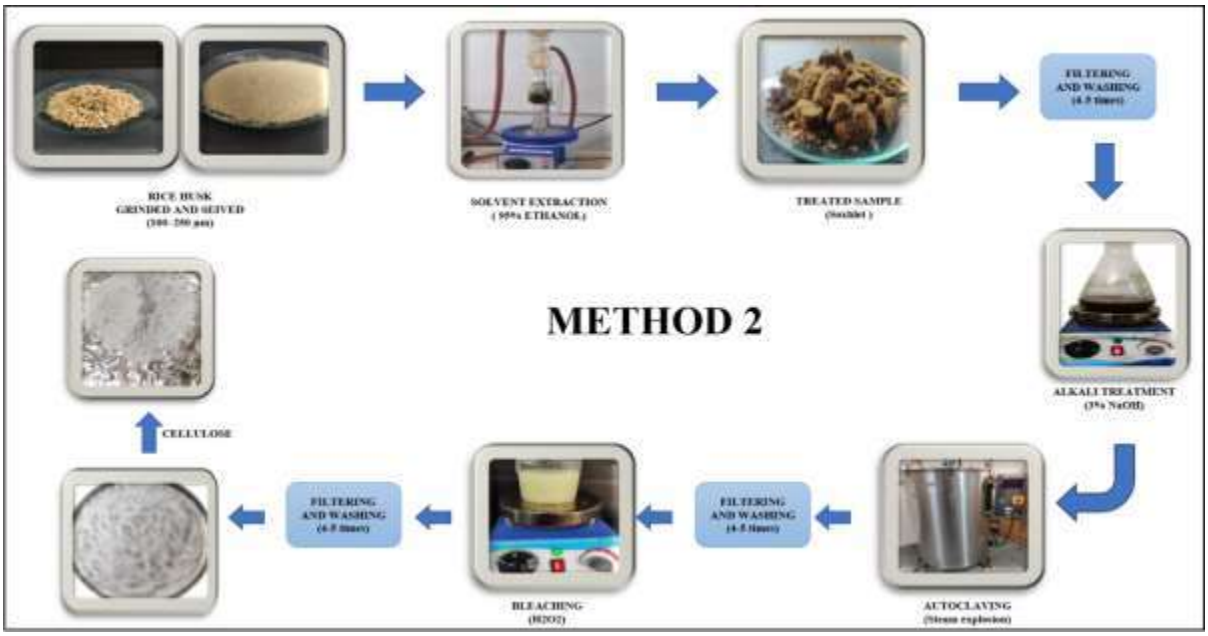

Figure 2. The process of the extraction of cellulose from waste rice husk by Ethanol-Mediated Solvent–Alkali–Autoclave-Assisted Extraction and bleaching method

2.2.2. Approach 3 (Thermal–Methanol/Benzene–Alkali–Autoclave-Assisted Extraction and Peracetic acid bleaching)

Cellulose was extracted from rice hulls/rice husk (RH) using thermal pretreatment, Soxhlet dewaxing, alkali treatment, autoclave assisted treatment and bleaching. Initially, 20 g of dried and sieved RH powder was treated with 100 mL distilled water at 100 °C for 1 h. The sample was filtered and then dried in a hot-air oven for 24 h after treatment. The dried thermally treated RH powder was then subjected to Soxhlet extraction with Ethanol: Benzene solvent mixture in the ratio of 1:2 (v/v). The extraction was performed for 6–7 h under reflux conditions at about 75–80 °C. After Soxhlet extraction, the treated sample was recovered, washed to remove residual solvent traces and dried overnight in a hot-air oven at 60 °C for alkali treatment. The dewaxed RH sample was subsequently treated with 5% (w/v) sodium hydroxide (NaOH) solution at a ratio of 1:20 g/mL of sample to solution. The suspension was stirred mechanically and heated to 45 °C for 6–8 h. The alkali treatment was followed by autoclave-assisted treatment of the alkaline suspension at 121 ± 2 °C and 15 psig for 15-20 min per cycle. The autoclaving was repeated twice to improve the disruption of the compact lignocellulosic matrix under the high temperature and high pressure steam conditions. Upon completion of the alkali–autoclave treatment, the resulting fibres were separated by filtration and thoroughly washed with distilled water until the filtrate was approximately neutral (pH 6–7). The washed alkali-treated fibres were bleached using a peracetic acid based bleaching solution prepared by mixing acetic acid, hydrogen peroxide and distilled water in a volume ratio 50:38:12. Acetic acid 125 ml, hydrogen peroxide 95 ml and distilled water 30 ml were mixed carefully for 250 ml of bleaching solution. Bleaching treatment was carried out at 60 °C for 6 h under constant stirring. If required, the bleaching step was repeated once with fresh solution to improve fibre whiteness and remove residual lignin and chromophoric impurities. The fibres were separated by vacuum filtration after bleaching and washed repeatedly with distilled water until the pH of the filtrate was 6–7. The bleached cellulose fibres were then dried in a hot-air oven at 35–45 °C for 12–24 h or until a constant dry weight was obtained. Finally, the dried cellulose fibres were collected and stored in clean airtight containers and labelled as RHC3 (Ishida et al., 2025; Johannes et al., 2025; Sharma et al., 2022; El-Din Al-Mofty et al., 2023) (Figure 3).

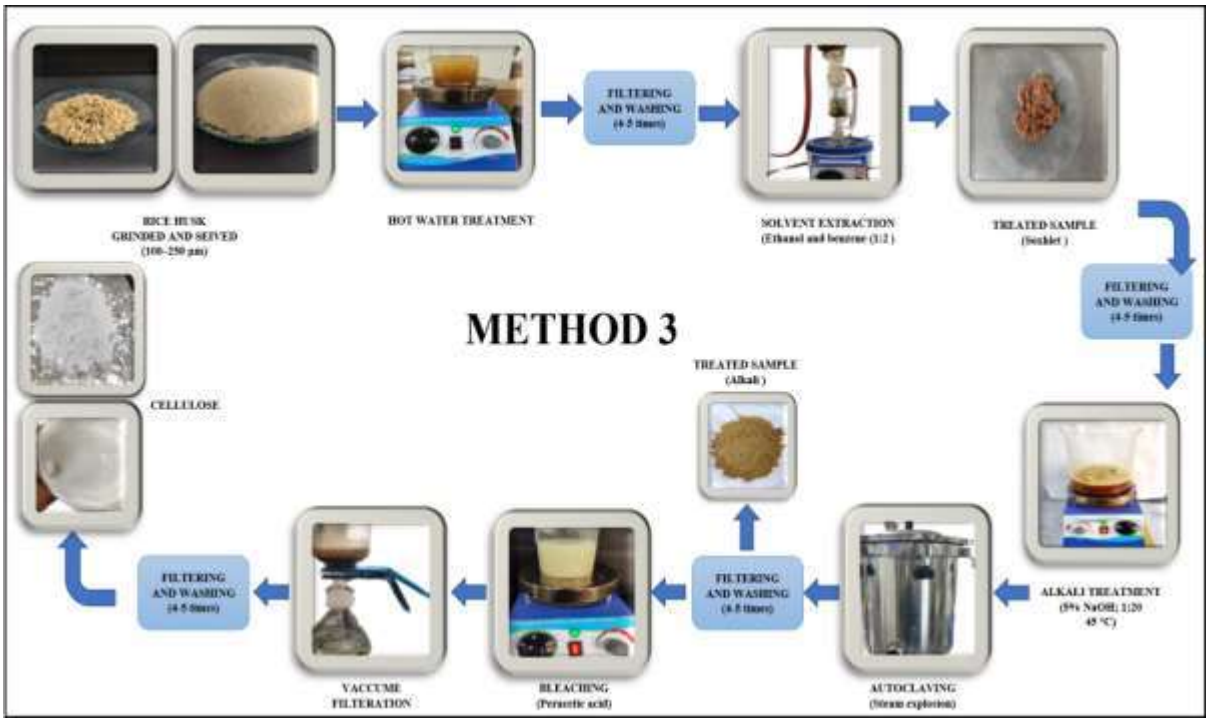

Figure. 3. The extraction and purification process of cellulose from waste rice husk by Thermal–Methanol/Benzene–Alkali–Autoclave-Assisted Extraction and Peracetic acid bleaching method

2.3. Physiochemical Characterizations

2.3.1. Quantitative estimation of extracted cellulose:

The extraction products were further evaluated by the Updegraff-Anthrone technique to obtain the percentage of cellulose in the extracted products according to the literature protocols Sequential alkali delignification with NaOH and peracetic acid was used to get cellulose by varying concentrations of acid-alkali. Three synergizing strategies that produced varying amounts of cellulose contents were tested. Under quantitative analysis firstly the extraction yield will be calculated (Figure 4)

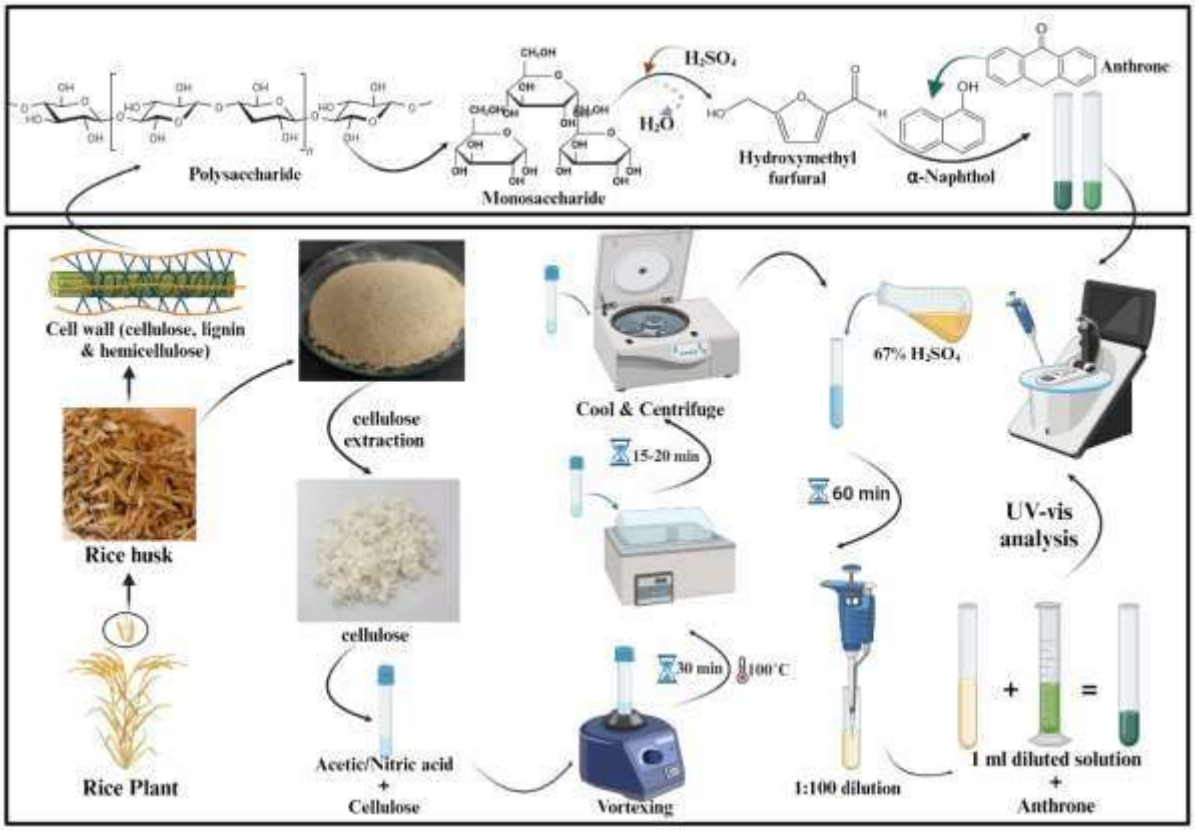

Figure 4. Schematic representation of Updegraff-Anthrone method showing the estimation of cellulose purity.

a. Extraction Yield (%):
This tells how much solid cellulose-rich material you recovered from rice husk after the extraction.

$$\text{Extraction yield (\%)} = \frac{\text{dry weight of extracted cellulose}}{\text{dry weight of original rice husk}} \times 100$$

b. Sample preparation:
10mg of each sample was soaked in 3 mL of acetic-nitric acid reagent and subjected to a boiling water bath of 100°C in 30min. Cooling occurred after which the samples were centrifuged within 15-20 min with the supernatant being discarded. This residue was first rinsed with 2-3 mL of distilled water and then 10 mL of 67% H_2SO_4 was added in ice bath and allowed to incubate at room temperature (60 min) to dissolve all the cellulose. Dilute 1ml or 0.1ml of this solution to 100 ml or 10ml. Vortex it and to 1ml of this diluted solution add 10 ml Anthrone heat it in boiling water bath for 5-10 min under observation. The absorbance at 630 nm was recorded in the UV-Visible spectrophotometer, after cooling. A standard calibration curve was used to compute the concentration of the cellulose (Gong et al., 2025)

c. Preparation of Standard curve:
A glucose stock solution of 1 mg/mL was used to create a glucose standard curve. To get glucose concentrations between 0.1 and 1.0 mg/mL, various quantities of this stock were pipetted into sterile test tubes. The final volume in each test tube was made up to 1.0 mL using distilled water. A blank was prepared using 1.0 mL distilled water without glucose. After preparation of the standards freshly prepared anthrone reagent was added to each test tube, including the blank. The tubes were mixed carefully and heated in a boiling water bath for 10-20 minutes to allow colour development. After heating, the tubes were cooled to room temperature, and the absorbance was measured at 620 nm against the reagent blank using a UV–Vis spectrophotometer. A standard curve was plotted by taking glucose concentration (mg/mL) on the X-axis and absorbance at 620 nm on the Y-axis.(Liu et al., 2026; Zhu et al., 2026)
Standard curve equation

$$y = mx + c$$

c. Calculate Cellulose purity percentage:

$$\text{Purity \%} = \frac{x \times 0.90 \times V \times Df}{W} \times 100$$

where:
- x = glucose concentration or glucose amount calculated from the absorbance
- y = absorbance of unknown sample
- c = intercept of standard curve
- m = slope of standard curve
- 0.90 = glucose-to-cellulose conversion factor
- V = total final volume of hydrolysed sample solution, mL
- DF = dilution factor
- W = weight of sample taken for the estimation, mg

2.3.2. Solubility assessment of extracted cellulose
The solubility behavior of extracted cellulose was evaluated in different solvents, such as methanol (CH_3OH), pyridine (C_5H_5N), sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), and dimethyl sulfoxide (DMSO). For each test, 10 mg of cellulose was added to 10 ml of solvent and vortex for 10-15 min. Solubility was analysed qualitatively and the results were noted in tabular form as mentioned earlier (Anamika et al.,2023).

2.4. Morphological, Structural and Crystalline analysis of extracted cellulose:
2.4.1. Scanning Electron Microscopy (SEM) analysis:
The surface morphology of the samples of untreated rice husk and extracted cellulose (RHC I, RHC II, and RHC III) was analyzed in a Coxem EM-30 scanning electron microscope. The samples were put on carbon tape in a vacuum and sputter-coated by a thin layer of gold on both sides to enhance conduction, especially in powdered samples. The images of SEM were taken at different magnifications at an accelerating voltage of 20 kV (Hafid et al.,2021).
2.4.2. Fourier-Transform Infrared Spectroscopy (FTIR) analysis
The FTIR spectroscopy was used to detect functional groups and verify the chemical structure of extracted cellulose. Spectra were recorded using a Cary 630 FTIR spectrometer (Agilent Technologies) was employed to perform spectral analysis. The samples were collected at 4000-400 cm^{-1} (resolution=2 cm^{-1}) and typical absorption bands were measured to determine the purity of the cellulose and the elimination of non-cellulosic substances (Sharma et al.,2023).
2.4.3. X-ray Diffraction (XRD) analysis
An X-ray diffractometer, Rigaku SmartLab, with the Cu Kα radiation (λ = 1.5405 A) was used to analyze the crystalline structure of the extracted cellulose. The fine ground samples were analysed in a 2θ range of 10°-80° with a step size of 0.02° and dwell time of 2 s/step. XRD patterns were applied to determine crystalline phases of cellulose, and also to determine the integrity of the structure after extraction. Vu et al. (2024)

The crystallinity index (CrI) was calculated using the Segal method from following equation:

$$\text{CrI}\ (\%) = \frac{I_{200} - I_{am}}{I_{200}} \times 100$$

Where, I_{200} = main peak

I_{am} = amorphous region

2.4.4. Statistical Analysis

All extraction experiments were conducted in triplicate (n=3), and they are reported as mean value ± standard deviation (SD). The standard descriptive statistical analysis was used to evaluate the experimental data. The OriginPro software was used to produce graphical representations. There were no inferential statistical comparisons in the study since the research was aimed at optimizing the methods and comparing them rather than hypothesis testing.

3. RESULTS AND DISCUSSION

3.1. Comparative analysis of Extraction strategies

Cellulose, hemicellulose, lignin, silica, waxes, and other extractives make up the majority of rice husk, a complex lignocellulosic agricultural residue. Consequently, in order to extract cellulose from rice husk, a series of treatments that can eliminate non-cellulosic components while maintaining the cellulose backbone are needed. In the current study, cellulose was extracted from rice husk using three different methods; the resulting samples were designated RHC1, RHC2, and RHC3. The degree and order of pretreatment, solvent extraction, alkali concentration, autoclave assistance, and bleaching system were the primary areas where the three methods varied. It is anticipated that these variations will affect the extracted cellulose's purity, yield, whiteness, crystallinity, and shape**.** The effectiveness of the extraction process of cellulose, using rice husk, highly relied on the sequence and intensity of pre-treatment, delignification, and bleaching steps. Quantitative analysis using the Updegraff-Anthrone method revealed a progressive increase in cellulose yield (%) among all three strategies. RHC-I (Approach I) yielded approximately 35.04 ± 2.4 mg/g which makes around 30-45% cellulose while, RHC II (Approach II) yielded 47.54 ± 1.4 mg/g (60-70% cellulose), and finally the RHC III (Approach III) achieved the highest yield of 70.72 ± 3.2 mg/g gives around 85-90% extracted cellulose (Table 1). The highest yield from strategy III can be attributed to combined solvent pre-treatment, efficient delignification via alkali treatment, and enhanced impurity removal through multiple bleaching steps. These results align with previous studies where combined chemical and thermal pre-treatment can enhance extraction efficiency (Popa et al.,2025). The main chemical process that extracts lignin from lignocellulosic biomass in an alkaline environment is the cleavage of α- and β-aryl ether and ester bonds between lignin and xylan, which increases the porosity of the biomass and consequently delignification (Singh et al.,2014).

In Approach 1, rice husk powder was treated with 1% NaOH under ultrasound assistance at 40 kHz and moderate temperature. Ultrasonication helps disrupt the compact lignocellulosic structure through cavitation, improving alkali penetration and removal of surface impurities, waxes, hemicellulose, and some lignin. However, due to the low NaOH concentration and mild conditions, complete removal of lignin, silica, and strongly bound hemicellulose may not occur. Therefore, RHC1 is expected to represent partially purified cellulose. The following alkaline hydrogen peroxide bleaching step further removes residual lignin and improves whiteness, while being greener than chlorine-based bleaching. Overall, this Approach can be considered a mild, greener and less chemically intensive method. Its major advantage is the use of low NaOH concentration and hydrogen peroxide bleaching. However, its limitation is that the mild alkali treatment may not be sufficient to achieve maximum delignification and cellulose purification. Therefore, RHC1 may represent a moderately purified cellulose sample with lower whiteness, lower crystallinity and more residual impurities compared with RHC2 and RHC3**.** Vu et al. (2017) used ultrasound-assisted alkaline treatment for lignin and cellulose extraction from rice straw and reported that ultrasound improves extraction efficiency by disrupting the lignocellulosic structure, Gavrila et al. (2024) based on saw dust, his work also supports the mechanism of mild alkaline treatment assisted by ultrasound for lignocellulosic biomass pretreatment. Kininge et al. (2021) showed ultrasound can intensify alkaline delignification by improving lignin and hemicellulose removal from lignocellulosic biomass. It supports your explanation of cavitation and improved NaOH penetration. The pre-treatment method was studied to enhance delignification and cellulose fiber extraction from miscanthus biomass, as reported by Singh et al., in their study. After 15 minutes of sonication, the highest cellulose concentration recorded was 47.8% (Singh et al.,2022). A similar type of study used alkali NaOH to extract cellulose, but they did not use ultrasonic assistance or bleaching using a buffer solution of acetic acid and aqueous chlorite. Despite this, they were able to recover 96% of refined cellulose, which is higher than this, but the chlorite made the procedure less ecologically friendly (Johar et al.,2012). Although the cellulose content of the extracted cellulose is not explicitly stated in the study. Novia et al. (2022) studied **lignin removal from rice husk using hydrogen peroxide** and confirmed that H_2O_2-based pretreatment can remove lignin and alter rice husk morphology. Battegazzore et al. (2014) also used the acid-alkali method to extract cellulose from rice husk using KOH and H_2SO_4, followed by bleaching with $NaClO_2$, and obtained approximately 26% lower than that of the cellulose obtained by initial approach I.

Approach 2 Involved pure ethanol Soxhlet dewaxing, 3% NaOH alkali treatment, autoclave assistance, and alkaline hydrogen peroxide bleaching. The ethanol dewaxing step removes surface waxes, oils, pigments, and extractives, improving the accessibility of NaOH to the rice husk matrix. Compared with Approach 1, the use of

a higher NaOH concentration at 75 °C provides stronger removal of hemicellulose, lignin, and silica-associated impurities. Autoclaving further enhances fibre swelling and disrupts the compact lignocellulosic structure, allowing better release of non-cellulosic components. The alkaline hydrogen peroxide bleaching step removes residual lignin and improves cellulose brightness. Therefore, RHC2 is expected to show higher purity, better whiteness, cleaner morphology, and improved crystallinity than RHC1, although this method requires more processing steps and energy input. Rezende et al. (2011), uses 95% ethanol for 6 h in a Soxhlet system to remove extractives from lignocellulosic biomass before chemical analysis/treatment. Johannes et al. (2025) studied 3% NaOH pretreatment of rice husk at high temperature in an autoclave system and reported significantly improved lignin removal. Wang et al. (2016); Novia et al. (2022); Vu et al. (2024) in their work showed H_2O_2 or $NaOH/H_2O_2$ treatment for lignin and hemicellulose removal from rice husk. Overall, this Approach had shown better performance due to the inclusion of solvent dewaxing, and autoclave-assisted delignification. High-pressure steam treatment during autoclaving probably favoured partial disruption of fibers and increased solubilization of lignin, leading to increased cellulose yield (%). However, residual non-cellulosic components were still present which restricted maximum purity (figure 5).

Figure 5. Cellulose obtained from Rice Husk from Approach I, II & III. (a) These were further represented as Rice Husk Cellulose-I (RHC-I), (b)Rice Husk Cellulose-II (RHC-II) & Rice Husk Cellulose-III (RHC-III)

Approach 3 was the most intensive cellulose extraction method among the three approaches. It involved thermal pretreatment, Soxhlet extraction using an ethanol/benzene solvent mixture, 5% NaOH alkali treatment, repeated autoclave-assisted treatment, and peracetic acid bleaching. The initial hot-water treatment at 100 °C helps soften the rice husk structure, remove water-soluble impurities, and improve chemical accessibility. Soxhlet extraction with ethanol/benzene removes waxes, oils, pigments, resins, and hydrophobic extractives, allowing better penetration of alkali into the lignocellulosic matrix. Jjj The 5% NaOH treatment provides stronger removal of hemicellulose, lignin, and silica-associated impurities compared with Approaches 1 and 2. It also promotes fibre swelling, breaks lignin–carbohydrate linkages, and dissolves amorphous non-cellulosic components. Repeated autoclaving further disrupts the rigid rice husk structure through high temperature and pressure, improving fibre opening, delignification, and cellulose separation. The peracetic acid bleaching system, prepared from acetic acid and hydrogen peroxide, acts as a strong oxidizing agent and removes residual lignin and chromophoric impurities more effectively than hydrogen peroxide alone. Therefore, RHC3 is expected to show the highest whiteness, cellulose purity, fibre separation, and crystallinity, with the lowest residual lignin content. These results consistent with earlier reports that show the multi-step chemical-thermal pretreatments can considerably increase cellulose extraction in rice husk and other agricultural wastes. (Rojas et al.,2024)

The three extraction approaches show a progressive increase in treatment severity from RHC1 to RHC3. RHC1 was produced using mild ultrasound-assisted alkali treatment and hydrogen peroxide bleaching. RHC2 involved ethanol dewaxing, stronger alkali treatment, autoclaving and hydrogen peroxide bleaching. RHC3 involved the most intensive sequence, including thermal pretreatment, solvent dewaxing, stronger alkali treatment, repeated autoclaving and peracetic acid bleaching. Based on the treatment conditions, RHC1 is expected to have the lowest cellulose purity among the three samples, but it is the simplest and least chemically intensive method. RHC2 is expected to show an intermediate to high level of purification due to ethanol dewaxing, stronger alkali treatment and autoclave-assisted fibre disruption. RHC3 is expected to show the highest degree of delignification, fibre separation, whiteness and crystallinity because of stronger chemical treatment and peracetic acid bleaching.

The expected order of cellulose purification can therefore be described as:

RHC1 < RHC2 < RHC3

This order can be justified by the progressive removal of waxes, extractives, hemicellulose, lignin and silica. RHC1 may retain a higher amount of residual lignin and hemicellulose because of the mild alkali condition. RHC2 should show improved removal of non-cellulosic components due to ethanol dewaxing and autoclave assistance. RHC3 should show maximum removal of impurities due to the combined effects of thermal pretreatment, stronger solvent extraction, higher NaOH concentration, repeated autoclaving and peracetic acid bleaching. (Figure5).

3.2. Quantitative Estimation of Cellulose

Cellulose content was estimated by the method of Updegraff and anthrone based colorimetric determination. In this method, noncellulosic components were removed with Updegraff reagent and the remaining cellulose-rich residue was hydrolysed and quantified as glucose equivalents using a glucose standard curve. Here, lignin, hemicellulose, and xylosans are extracted using an acetic acid/nitric acid reagent. The anthrone reagent is used to determine the composition of the residual cellulose after it has been dissolved in 67% H_2SO_4. The formation of blue-green colour after reaction with anthrone reagent confirmed the presence of carbohydrate derived compounds in the hydrolysed sample. As the concentration of glucose increased, the colour intensity also increased. This indicated that the prepared glucose standards were suitable for the calibration range for the estimation of cellulose in the unknown sample.

$$\text{Carbohydrate} + \text{Anthrone reagent} \xrightarrow[\text{heat}]{\text{acid}} \text{Blue-green complex}$$

Overall reaction for cellulose estimation:

$$\text{Cellulose} \xrightarrow[\text{acid hydrolysis}]{\text{heat}} \text{Glucose} \xrightarrow[\text{conc. } H_2SO_4]{\text{dehydration}} \text{5-Hydroxymethylfurfural} \xrightarrow[\text{anthrone}]{\text{acidic medium}} \text{Blue-green complex}$$

Cellulose is hydrolysed into glucose units. Glucose is dehydrated to 5-hydroxymethylfurfural under the influence of concentrated sulphuric acid and heat. This intermediate then reacts with anthrone and forms a stable blue green coloured complex. The intensity of this colour is proportional to the glucose concentration in the sample and is measured spectrophotometrically at 620 nm. This glucose equivalent from the standard curve is thus converted to cellulose content using the anhydroglucose correction factor. (Souza et al.,2022). The three strategies presented here demonstrated differing cellulose percentages as determine by the Updegraff method: 30–45% for the first method, 60–70% for the second, and 75-80 % for the third (Table 2). The percentage of the cellulose content in Strategy III was higher than that of Strategy I and Strategy II, which proves that the more the steps of pretreatment and bleaching are performed progressively, the higher the recovery of cellulose will be. Strategy III was the only one that showed the highest percentage of cellulose (75-80 %), which confirmed its usefulness as an effective extraction protocol. These findings are like those that have been found in published data of optimized methods of cellulose extraction of rice husk using combined chemical and thermal methods (Onovo et al., 2025) (Table 1).

Table 1. Showing the extraction yield in grams and % and estimated purity %.

Samples	Rice husk powder	Extraction yield		Purity %
RHC 1	20 gm	5.20 ± 0.12 g	26.05 ± 0.60 %	30.75 ± 2.4 %
RHC 2	20 gm	10.8 ± 0.18 g	53.75 ± 0.90 %	61.82 ± 1.4 %
RHC 3	20 gm	13.8 ± 0.22 g	69.15 ± 1.10 %	78.21 ± 3.2 %

3.2. Solubility Assessment of Extracted Cellulose:

The solubility behaviour of extracted cellulose gives an insight into the structural integrity as well as crystallinity. It was observed that all RHC samples were insoluble in water, methanol and DMSO otherwise, which is typical of strong intra and intermolecular hydrogen bonding associated with native cellulose. However, cellulose exhibited solubility in dilute NaOH (<5% at 4°C), concentrated H_2SO_4(≥67%), and pure pyridine solution, consistent with previous reports (Hudson et al.,1980). While other authors have demonstrated efficient extraction using acid-chlorite or KOH treatments (Zhang et al.,2010). This study emphasizes eco-friendliness and method comparison (Table 2). These solvents disrupt hydrogen bonding within the cellulose structure, enabling dissolution. These findings support previous studies that cellulose has a highly crystalline structure with significant intra and intermolecular hydrogen bonding, making it water insoluble. It can dissolve only if these hydrogen bonds are successfully broken. Cellulose swells and becomes soluble at room temperature or low temperatures when the acid concentration rises; this is because of the hydrolysis process. Cellulose hydrolyzes and dissolves more readily when the acidic treatment and temperature increased (Xiang et al.,2003). Overall, It is interesting to note that RHC-III dissolved in NaOH and concentrated sulphuric acid quicker and more consistently than in RHC-I and RHC-II. The behaviour indicates an increased level of cellulose exposure and a decreased level of lignin shielding, which is in line with the greater level of delignification that was attained in Strategy III.

The results of the solubility patterns are consistent with the findings of the earlier literature that has indicated selective dissolution of high-purity cellulose under alkalinic and acidic standards (Kalita et al.,2015). The observed solubility behaviour also highlights the potential for further chemical modification of cellulose. In particular, dissolution of cellulose in suitable solvent systems can facilitate homogenous-phase reactions, enabling the synthesis of other cellulose derivatives with improved functional properties. Such modifications are important for developing advanced materials with specific applications. Additionally, efficient dissolution and processing of cellulose offer some advantages such as scalable production and high value applications. However, challenges

like solvent recovery, process efficiency, and optimization of dissolution conditions remains. Therefore, further studies are required in this area. (Table 2)

Table 2. Shows solubility of the extracted cellulose in different solvents.

Solvents (10ml)	**Sulphuric acid (H_2SO_4**	**Sodium Hydroxide (NaOH)**	**Pyridine**	**Methanol**	**DMSO**
5%	Insoluble	**Soluble (4°C)**	Insoluble	Insoluble	Insoluble
10%	Insoluble	**Fairly soluble (4°C)**	Insoluble	Insoluble	Insoluble
25%	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
50%	Insoluble	Insoluble	Insoluble	Insoluble	Insoluble
75%	**Soluble**	Insoluble	**Fairly soluble**	Insoluble	Insoluble
100%	**Soluble**	Insoluble	**Soluble**	Insoluble	Insoluble

3.3. Morphological & Structural Analysis of Extracted Cellulose:

Cellulose fiber recovered from rice husk can undergo certain notable modifications because of acid-alkali treatments (figure 6). These modifications include a reduction in fiber dimensions, an increase in aspect ratio, and the exposure of microfibrils. Furthermore, surface characteristics are altered, resulting in improved porosity and increased roughness (Sharma et al.,2023). Changes in crystallinity are also observed, potentially including crystal structure transformations and an increase in the degree of crystallinity. Comprehensive characterization is performed to confirm modifications.

SEM Analysis

Cellulose fiber recovered from rice husk can undergo certain notable modifications because of acid-alkali treatments (figure 6). These modifications include a reduction in fibre dimensions, an increase in aspect ratio, and the exposure of microfibrils. Furthermore, surface characteristics are altered, resulting in improved porosity and increased roughness (Sharma et al.,2023). Changes in crystallinity are also observed, potentially including crystal structure transformations and an increase in the degree of crystallinity. Comprehensive characterization is performed to confirm modifications.

The cellulose samples extracted exhibited different morphological changes after chemical treatment. RHC 1 (Figure. 8 (c,d)) shows a partially disrupted compact rice husk structure. The surface appears more fibrous than that of the raw rice husk. Long fibre bundles are visible, and the cellulose fibrils are partly separated. In some areas, removal of non-cellulosic components may be incomplete and still appear dense and aggregated. The existence of bundle and layered structures showed that cellulose was exposed and the fibres were not completely separated.

The morphology of RHC 2 in Figure. 8 (e,f) is more open and loosened than that of sample 1. The micrographs showed irregular, porous and highly entangled fibrillar structures. The fibre network seems to be less dense, suggesting that there is more removal of lignin, hemicellulose and surface impurities during treatment. The existence of separated and curled fibrils indicates that the chemical treatment was more efficient to disrupt the lignocellulosic matrix. The higher porosity and roughness of sample 2 may result in a better accessibility of the surface, which is beneficial for further modification, adsorption or composite formation.

As seen in Figure. 8 (g,h), RHC 3 shows the most distinct fibrous morphology among the extracted cellulose samples. SEM images showed long, aligned and well-separated bundles of cellulose fibres with a rough surface. Sample 3 shows more evident fibrillation and better exposure of cellulose fibres in comparison with RHC 1 and RHC 2. The compact structure of raw rice husk is almost destroyed, which suggests that the amorphous components such as lignin and hemicellulose have been effectively removed. The longer, individual fibrils indicate a successful cellulose extraction and suggest that this treatment condition yielded a cellulose with a higher degree of structural purity and surface accessibility.

So, the SEM results confirm the change of raw rice husk from a compact and impurity-covered structure to a rough porous and fibrillar cellulose-rich material. The progressive increase in fibre separation from RHC 1 to RHC 3 shows that the extraction method has a strong influence on the morphology of the recovered cellulose. Among the three extracted cellulose samples, RHC 3 exhibited the most exposed, elongated and separated fibrillar bundles. This morphology indicates the effectiveness of the extraction process to remove the non-cellulosic components and expose the microstructure of the cellulose. However, SEM analysis mainly provides

morphological evidence and should be supported by FTIR, XRD for more chemical structure changes. (Oliveira et al.,2017) (Figure 6)

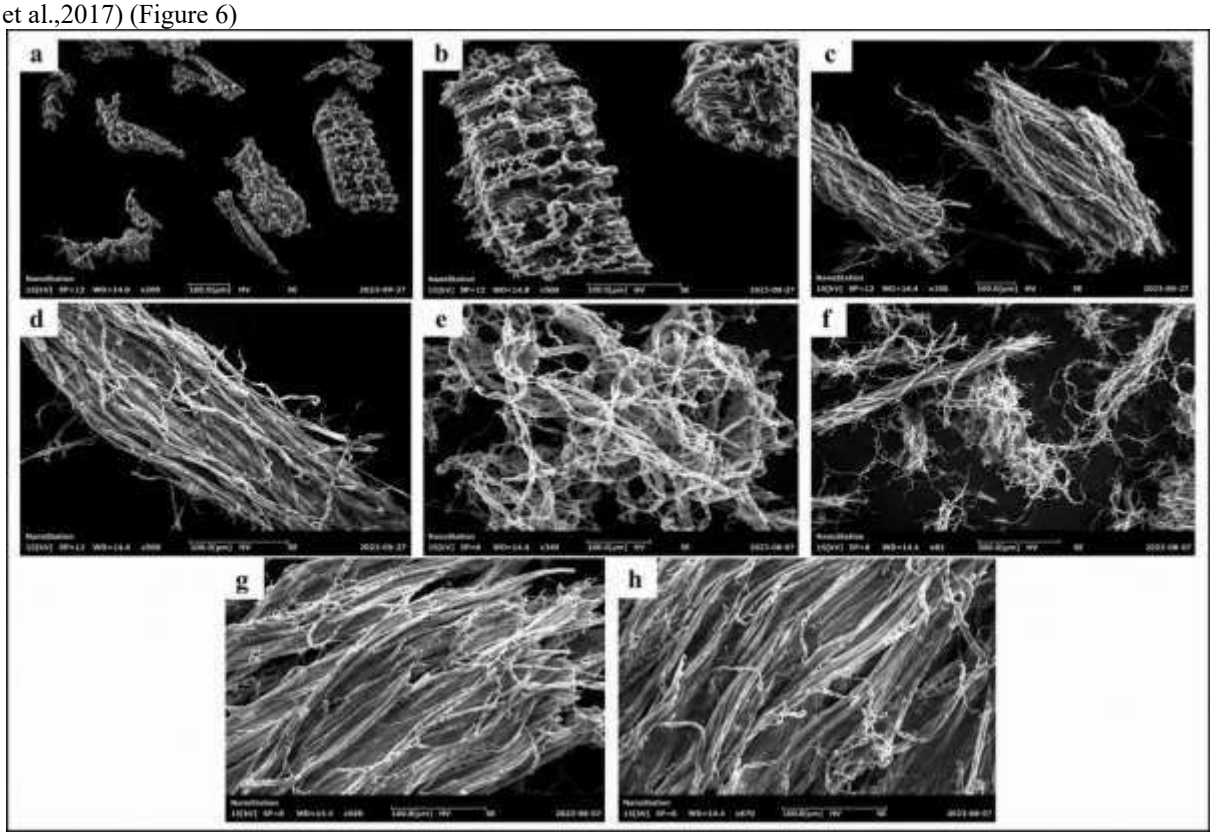

Figure. 6. The scanning electron microscopic (SEM) images of the rice husk cellulose extracted using all the different approach strategies. Figure 8(a-b) indicates the untreated rice husk. Figure 8(c-d) indicates the RHC-I obtained through the approach Strategy-I. Figure 8(e-f) indicates the indicates the RHC-II obtained through the approach strategy-II. Figure 8(g-h) indicates the RHC-III obtained through the approach strategy-III. The obtained images were in compatibility with other cellulose images represented in the cited literature

FTIR Analysis:

The primary cellulose functional groups are present in the extracted rice husk cellulose, according to an FTIR comparison between RHC III and commercial cellulose. The cellulose backbone was successfully preserved after extraction, as seen by the large O–H stretching band, C–H stretching band, CH_2 deformation band, strong C–O/C–O–C fingerprint region, and β-glycosidic band at 895.92 cm^{-1}. Effective hemicellulose removal is indicated by the lack of a prominent 1730–1740 cm^{-1} hemicellulose carbonyl band. Nonetheless, low-wavenumber bands around 800 cm^{-1} and faint bands around 1551–1514 cm^{-1} indicate that trace lignin and possibly silica/mineral remnants are still present in RHC III. Consequently, RHC III can be characterised as a cellulose-rich extracted material with FTIR properties like those of commercial cellulose, but with trace amounts of contaminants produced from rice husks.

The O–H stretching vibration of the hydroxyl groups in cellulose is represented by the broad absorption band in RHC III at 3333.78–3272.14 cm^{-1}. In the same area, commercial cellulose likewise displays a wide band. This resemblance demonstrates that Sample 3 has a lot of hydroxyl groups and strong hydrogen bonds both within and between molecules, which are traits of cellulose.

The C–H stretching vibrations of the –CH and $–CH_2$ groups in the cellulose glucopyranose unit are represented by the bands at 2938.90, 2906.19, 2864.37, and 2843.94 cm^{-1} in RHC III. This band is also visible in commercial cellulose in the same area. As a result, the extracted cellulose retains its aliphatic cellulose backbone. FTIR investigations of cellulose and lignocellulosic fibres indicate similar cellulose bands about 2900 cm^{-1}.

The lack of a prominent band in RHC III about 1730–1740 cm^{-1} is an extremely significant finding. This area is typically attributed to the C=O stretching of hemicellulose's acetyl and uronic ester groups. This band's lack of visibility suggests that the majority of the hemicellulose was eliminated during the bleaching and alkali treatments. This is consistent with pretreatment experiments on rice husks, which show a drop in the carbonyl band about 1735 cm^{-1} following hemicellulose removal. The band at 1655.04 cm^{-1} is mostly caused by adsorbed water's H–O–H bending. This band is typical since cellulose is hydrophilic. The minor peak/shoulder at 1692.85 cm^{-1} could be caused by mild oxidation, absorbed moisture, or traces of residual carbonyl-containing compounds. This area looks a little more complicated in RHC III than in commercial cellulose, indicating that there might be some moisture or leftover non-cellulosic particles in the extracted cellulose. Both RHC III and commercial cellulose exhibit the bands at 1452.44 and 1426.53 cm^{-1}. These are attributed to the cellulose's CH_2 scissoring vibration. The organised or crystalline portion of cellulose is similarly linked to the band at 1426–1430 cm^{-1}. Its existence

in RHC III shows that, following chemical treatment, the extracted cellulose maintained a crystalline cellulose structure. In RHC III, cellulose ring vibrations, C–H bending, and O–H in-plane bending are located between 1376.80 and 1314.45 cm^{-1}. These bands are likewise found in commercial cellulose, indicating that the extracted sample still has the cellulose's glucose ring structure. The C–O–C asymmetric stretching of the β-1,4-glycosidic bond in cellulose, the bands at 1160.30 and 1106.82 cm^{-1} are crucial. The same fingerprint region is seen by commercial cellulose. This demonstrates that RHC III has the distinctive cellulose glycosidic bridge structure. One of the most significant confirming bands in RHC III is located at 895.92 cm^{-1}. It is attributed to cellulose's β-glycosidic C–H deformation and C–O–C vibration. This demonstrates that β-1,4-glycosidic bonds exist between glucose units. In this area, commercial cellulose likewise displays a comparable band. This band is frequently found about 897 cm^{-1} in reported cellulose FTIR measurements. (Figure 7) (Table 3).

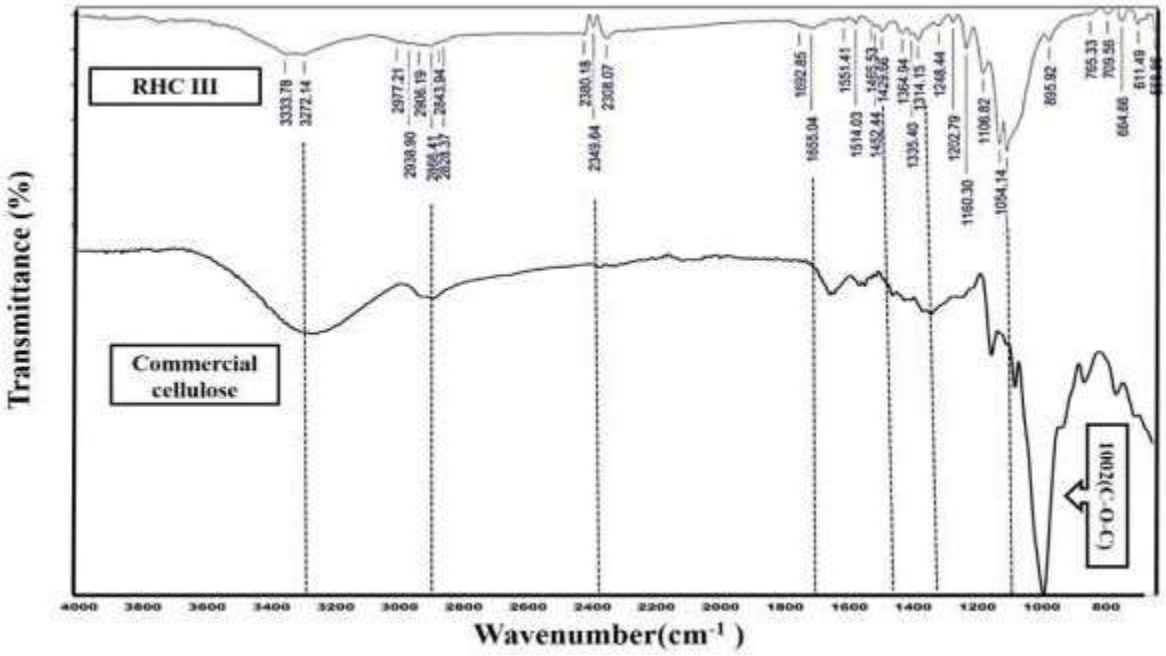

Figure 10. Fourier Transform Infrared Spectroscopy (FTIR)data shows signature peaks of the RHC-III obtained from the approach strategy-III, with the comparison of commercial cellulose, as this process yields the highest amount of extracted cellulose from the rice husk

Table 3. FTIR band analysis reports comparative analysis of the RHC 3 with the commercial cellulose

Region / band	RHC III	Commercial cellulose	Assignment	Comparative interpretation
3333–3272 cm^{-1}	Present	Present, broad	O–H stretching of cellulose hydroxyl groups	Confirms abundant cellulose –OH groups and hydrogen bonding. RHC III matches commercial cellulose well in this region.
2938–2843 cm^{-1}	Present	Present near 2900 cm^{-1}	C–H and CH_2 stretching of cellulose backbone	Confirms the glucopyranose ring structure of cellulose. Literature reports cellulose bands near 2900 cm^{-1}. (Md Salim et al., 2021)
~1730–1740 cm^{-1}	Not clearly present	Not present	C=O stretching of hemicellulose acetyl	Absence or very weak intensity indicates effective removal of hemicellulose. Literature assigns the 1735–1736 cm^{-1} band to hemicellulose/lignin ester carbonyls.(Md Salim et al., 2021)
1452 and 1426 cm^{-1}	Present	Present	CH_2 scissoring / cellulose crystalline band	Good match with commercial cellulose. The band near 1427–1430 cm^{-1} is associated with ordered/crystalline cellulose.(Md Salim et al., 2021)
1376–1335 cm^{-1}	Present	Present	C–H bending and O–H in-plane bending of cellulose	Confirms cellulose ring and glucose-unit vibrations. This region is commonly observed in cellulose spectra.
1160 and 1106 cm^{-1}	Present	Present	C–O–C asymmetric stretching of β -1,4-glycosidic bridge	Strong evidence of cellulose. Commercial cellulose also shows this region clearly.

				Literature reports cellulose bands around 1160–1165 cm^{-1}. (Md Salim et al., 2021)
1054 and 1030 cm^{-1}	Strongly present	Strongly present	C–O stretching and C–O–C pyranose ring vibration	This is the strongest cellulose fingerprint region. RHC III and commercial cellulose are very similar here.
895 cm^{-1}	Present	Present	β-glycosidic linkage of cellulose	Band confirms β-glycosidic linkage in the cellulose chain. Literature reports band at 897cm^{-1}.(Romruen et al., 2022)

XRD Analysis

The XRD analysis confirmed the crystalline structure of cellulose. The diffractogram of rice husk cellulose showed three diffraction peaks at angles 2θ of 14.95°, 22.56°, and 34.74°, which correspond to the 110, 200, and 040 crystallographic planes of cellulose respectively. These reflections are consistent with the typical diffraction pattern of native cellulose, indicating that the crystalline phase has been preserved during the extraction process. The prominent peak around 22.5° indicates a high degree of crystallinity, which is crucial for the mechanical strength and stability (Huang et al.,2015). The presence of the flat curve at 14.95 suggests the existence of amorphous regions present within the crystalline lattice, which is typical of semi-crystalline cellulose. The third diffraction peaks observed at 34.74 further confirm the ordered packing of cellulose microfibrils and support the identification of cellulose phase. The overall pattern does not indicate the presence of other components, suggesting that there is no regeneration occurred during the extraction process. Our studies align with the earlier studies on rice husk cellulose, where similar diffraction peaks were reported after alkali and bleaching treatments and also confirm that the extraction protocol in the third strategy was effective in removing lignin and hemicellulose without compromising the cellulose integrity. Diffraction peaks in the 22–23° range demonstrated the structure of cellulose and were typical of the original cellulose. The fact that there were no other diffraction peaks that would have indicated lignin or regenerated cellulose phases is confirmation that the native crystalline structure was maintained through the extraction process. The intensity of crystalline peak (I_{200}) at 22.4° and the amorphous intensity (I_{am}) at 18° were used to calculate the crystallinity Index (CrI) for RHC III and it has been found 78.2%, indicating a high degree of crystallinity. The advantages of the crystallinity of RHC-III can be explained by the successful extraction of amorphous hemicellulose and lignin components, which in turn supports the effectiveness of the optimized extraction strategy (Figure 11) (Suryanto et al., 2021; Samsalee et al. (2023))

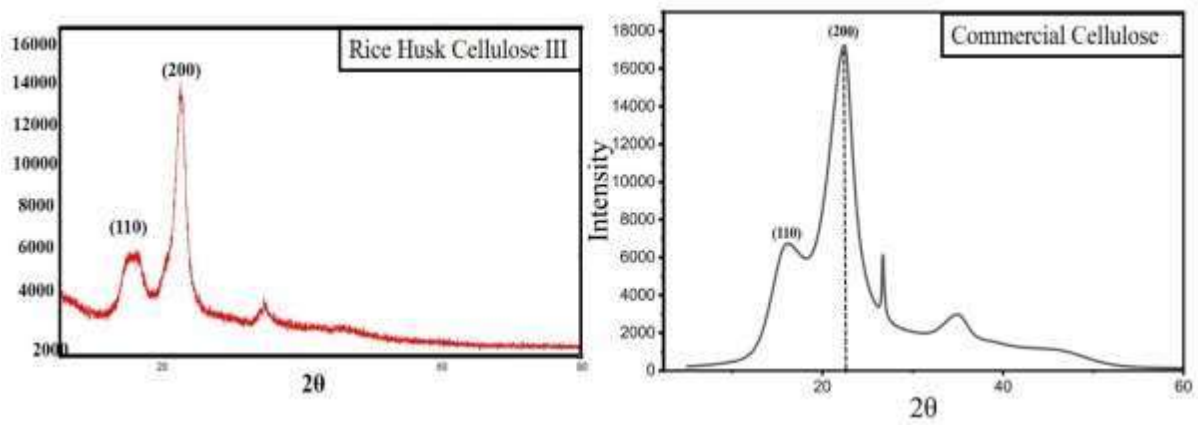

Figure 11. X-ray diffraction (XRD) of the extracted RHC-III obtained from the approach strategy-III, as this process yields the highest amount of extracted cellulose from the waste rice husk, with the comparison of Commercial cellulose. The peaks obtained by the XRD analysis indicate the lateral packing of cellulose chains (e.g., 2θ peak at 16°), and simultaneously, the 2θ peak at 22.5° is indicative of cellulose crystalline structure

4. CONCLUSION:

The combined results of the quantitative, spectroscopic, morphological, and structural analyses reveal that Strategy III has the most effective and sustainable path towards the extraction of high-purity cellulose in rice husk. Pre-treatment of the solvent, increased alkali delignification, fiber disruption using autoclave and bleaching without chlorine led to an increased yield, purity and crystallinity of cellulose. These characteristics render the extracted cellulose very applicable to biomedical materials and hydrogel as well as sustainable biopolymer composites. This study not only advances cellulose extraction methodologies but also reinforces the utility of rice husk as a cost-effective and renewable resource in green biopolymer development. Cellulose, a versatile biopolymer, can be sourced from a variety of plant and microbial origins, with rice husk identified as a particularly promising sustainable source for cellulose extraction. This interest stems from rice husks' abundant availability as an agricultural by-product and their inherent rich lignocellulosic composition, which makes it a potentially cost-

effective feedstock for cellulose production. However, the extraction of cellulose from rice husk is not without its challenges. Traditional methods for extracting cellulose from rice husk are often labour intensive and require multiple steps to obtain a high purity of cellulose. Furthermore, these methods often involve a complex sequence of multiple processing steps, each contributing to the overall complexity and cost of the extraction process (Chopra et al.,2022). A critical challenge in cellulose extraction from rice husk lies in the attainment of high-purity cellulose. The presence of non-cellulosic components, such as lignin and hemicellulose, can significantly compromise the quality and applicability of the extracted cellulose. In a recent comparative evaluation of three distinct extraction techniques assessed, the notable disparity was observed in the third method, which exhibited the highest cellulose extractability, achieving a purity level of 70-80% thereby establishing it as the most effective technique for cellulose extraction. In contrast, the first and second methods appear to retain a substantial amount of non-cellulosic components, resulting in comparatively lower purity of the extracted cellulose (Tredec et al.,2008). This limitation underscores the shortcomings of these methods in achieving high-quality cellulose yield, highlighting the importance of selecting an appropriate extraction technique to maximize cellulose purity and minimize the presence of unwanted impurities. This also emphasizes the future studies to explore scale-up potential and techno-economic assessment of the optimized protocol as well as integration of this cellulose into composite biomaterials.

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Authors contribution
All authors were contributed to the conception and design of the study. Chitranshi Sharma conducted the material preparation, data collection, analysis and initial draft writing. Iram Jahan contributed to manuscript preparation. All authors reviewed and revised earlier versions and edited the final version of the draft. The revised final manuscript was read and approved by all authors.

Data Availability
Due to copyright and originality of work, the data supporting the findings of the study are not openly accessible. However, the datasets may be provided by the corresponding author upon justified and reasonable request.

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