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Impact of Alkali Concentration in Delignification Treatment for Cellulose Extraction from Rice Straw

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Abstract

Rice straw is an abundant agricultural byproduct with great potential as a sustainable source of cellulose. This study investigates the effect of sodium hydroxide concentration on the extraction of cellulose from rice straw through alkali delignification. Sodium hydroxide concentrations ranging from 3% to 11% were applied at a constant temperature of 60 °C for 60 minutes. The objective was to assess the impact of alkali concentration on cellulose yield and structural properties. Results showed that increasing alkali concentration led to a reduction in cellulose yield. Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) confirmed the effective removal of lignin, hemicellulose, and impurities across all concentrations. Additionally, the crystallinity index (CI) increased from 47.85% to 51.37% as alkali concentration rose, indicating successful delignification. However, a balance must be considered, as lower alkali concentrations, while environmentally friendlier and yielding more cellulose, resulted in lower purity, with FTIR, SEM, and XRD analyses revealing residual impurities in the extracted cellulose. This eco-friendly process offers a promising method for converting agricultural waste into valuable cellulose, contributing to sustainable industrial practices.

Paper type: Research paper

Keywords: Cellulose, extraction, alkali delignification,rice straw.

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Introduction

In current times, the population size of living species is increasing, leading to a rapid escalation the consumption and demand for food. Subsequently, a million tons of rice is produced annually. Hence, rice plays an important role as the source of energy, essential vitamins, nutrients, carbohydrates, etc. Those elements inside the rice are important and essential for the human body. Furthermore, Malaysia stands as a significant producer of rice, with the as the average Malaysian citizen consumes 82.3 kg of rice per year, and an average of 3.7 metric tons (MT) of rice is produced per hectare of paddy field (Harun, Hanafiah, and Aziz 2021).

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Hence, rice is the staple food for most of the country in Asia, particularly in Malaysia, which provides us with an important portion of calories in our daily lives. For further illustration, the global rice production ranges from 650 to 975 million tons annually, yielding approximately 1 to 1.5 kg of residual rice straw per unit (Muliarta 2019). Consequently, the increasing amount of rice production will result in an increase of amount of residual rice straw, an inherent agricultural byproduct generated during harvesting. Unfortunately, improper waste management results in a rapid increase in the accumulation of residual rice straw worldwide. This is due to the high fiber concentration in its composition and the low biodegradability that causes it to build on the fields. To illustrate, this byproduct encompasses both rice husk (RH) and rice straw (RS) (Mahmod et al. 2024), with only a limited number of farmers that responsibly convert rice straw into organic fertilizer through composting. Regrettably, there are certain farmers that resort to open burning to simply dispose of the rice straw due to low cost of operation and simplicity in disposal methods. This unsustainable practice not only contributes to air pollution but also poses severe health risks to the public while worsening environmental issues (Kesari and Jamal 2017). Rice straw, in fact, qualifies as a renewable source characterized by its abundant cellulose content, falling within the category of lignocellulosic materials. Notably, it surpasses other agricultural products in cellulose concentration. Cellulose constituents are the primary component of rice straw, forming a dense structure composed of hemicellulose closely associated with lignin (Mahmod et al. 2024). Hence, there are various methods to extract and convert rice straw into valuable materials of cellulose, for instance, chlorine-free method, combined chemicals methods, enzymatic reaction, organosolv process, steam explosion, and more. Among these, combined chemicals method involving an alkaline treatment process has proven to be the most efficient and inexpensive method for hemicellulose removal, eliminating soluble lignin, waxes and other impurities present in plant cell wall (Al-Rajabi and Haan 2022).

Several studies have explored the use of alkali delignification with varying NaOH concentrations, demonstrating the flexibility of this method in processing lignocellulosic biomass. Ling et al. investigated delignification using NaOH concentrations ranging from 8–12 wt%, highlighting its effectiveness across a broad range (Ling et al. 2017). Similarly, Kargarzadeh et al. employed a 4 wt% NaOH solution for the delignification of kenaf fibers, illustrating the feasibility of lower concentrations in achieving sufficient lignin and hemicellulose removal (Kargarzadeh et al. 2012). Hafid et al treated rice husks with a 5%NaOH solution, demonstrating its applicability in extracting cellulose from agricultural waste (Hafid et al. 2021). Kaur et al. applied 5%(w/w) NaOH to rice straw, showcasing its potential for producing cellulose-rich fractions (Kaur et. al, 2023) . These studies justify the selected range of NaOH concentrations (3%–11%) in this study, as they align with established methodologies and highlight the adaptability of NaOH concentrations to different biomass types and treatment objectives.To ensure an energy-efficient and cost-effective approach, this study employed a delignification process at 60 °C for 60 minutes. These conditions were selected based on our prior optimization work, which demonstrated their effectiveness in achieving significant lignin removal without compromising cellulose yield. Compared to conventional methods reported in the literature, such as using 95 °C for 2 hours (Han and Geng 2023), 121 °C for 1 hour (Kaur et. al, 2023), and 120 °C for 45 minutes (Hafid et. al, 2021), the selected conditions offer a more sustainable alternative. This approach reflects the study's aim to develop environmentally friendly and energy-efficient cellulose extraction methods.

Although acid pretreatment can hydrolyse the hemicellulose by destroying the polymeric bonds, enhancing the availability of cellulose in rice straw, but it is not effective in dissolving lignin (Keskin et. al, 2019). Hence, the primary concern revolves around refining methods and techniques is to study the effect of alkaline treatment parameters for extracting cellulose from rice straw. This study specifically concentrates on study the effect of alkali concentration during alkaline treatment parameters to enhance the efficient removal of lignin, ultimately striving for a high yield of pure cellulose. Therefore, several experiments were performed by varying the alkali concentration at constant time and temperature. This study places a particular emphasize on examining the morphological and structural alterations of the extracted cellulose fibres using different concentration of alkali.

1 Materials and methods

1.1 **Materials**

Analytical grade formic acid (HCOOH) and hydrogen peroxide (H_2O_2) were used for acid treatment and were purchased from Sigma Aldrich (Burlington, MA, United State). Sodium hydroxide with analytical grade was used for the alkali treatment and were purchased from Merck (Darmstadt, Germany). Deionized water was obtained from the Faculty of Chemical Engineering and Technology's laboratory in Jejawi, Perlis. The rice straw was obtained from The Seed Sdn Bhd, Jalan Industri 1, Kawasan Perindustrian Gurun, 08300Gurun, Kedah.

1.2 Extraction of Cellulose from Rice Straw

The pretreatment process was adapted and modified from a research study conducted in our previous work (Al-Rajabi and Haan 2022; 2021; AL-Rajabi and Teow 2023b; 2023a; Al-Rajabi and Teow 2021). Firstly, the rice straw was washed, cut, and dried to remove the moisture content of the rice straw. The collected rice straw was then washed by fresh water to remove contamination like soil, dust, insect larvae, etc. Then, the rice straw was cut into smaller pieces, in about 5 cm length. Following, the rice straw was oven-dried at 60°C for 24 hours until constant weight is obtained. The rice straw was grinded into fine powder and sieved before packing inside an airtight, sterilized container to prevent contamination.
The acid hydrolysis was started by adding 10 g of the rice straw and soaked in 200 mL mixture of 20% v/v% formic acid and 10 v/v% hydrogen peroxide at volume/volume ratio (v/v) of 1:1. The mixture was then heated to 85°C for 2 hours using a hot plate with a magnetic stirrer (Al-Rajabi & Haan, 2022). Next, the delignification process was carried out by varying the alkali concentration (w/v%) (as shown in **Table 1**).

Table 1. Alkali concentrations (w/v%) used in the delignification process for cellulose extraction from rice straw.

Run	Alkali concentration (%)	Temperature (°C)	Time (min)
1	3		
2	7	60	60
3	11		

Following alkaline treatment, the solution was cooled down and filtered to eliminate the soluble lignin, as detailed by (Sharma et. al, 2023). Subsequently, inspired by the work of Al-Rajabi & Haan, (2022), cellulose fibers were undergone a bleaching process. These cellulose fibers were immersed in 10 v/v% hydrogen peroxide solution at 60°C for 90 minutes. The pH of the 10 w/v% hydrogen peroxide was adjusted to pH 9 using 10 w/v% NaOH. Then, the resulting white cellulose fibres were filtered, rinsed, and washing with deionized water until neutral pH is achieved. The insoluble fraction of cellulose fibres was gathered and dried in oven at 60°C for 24 hours. The dried weight of the extracted cellulose fibers was measured using a weighing balance to calculate the yield of cellulose extracted using **Equation (1)** below.

$$\text{Yield } (\%) = \frac{\text{m}'(\text{g})}{\text{m}_0\,(\text{g})} \times 100\% \quad (1)$$

Where m' is the final dry weight of the cellulose fibers whereas m_0 is the original weight before delignification of the cellulose fibers.

1.3 Characterization of Cellulose

Next, for the characterization of the cellulose using different alkali concentration, it was done using several approaches and apparatus which are the Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-Ray Diffraction Analysis (XRD), etc. The elaboration on the characterization and measurement of cellulose is discussed in section below.

1.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

Both the rice straw and the extracted cellulosic samples were analyzed using Fourier transform infrared spectroscopy (Nicolet 6700), operating in the transmittance range of 400 to 4000 cm^{-1}. This technique was employed to examine the structural characteristics of rice straw fibers and identify functional groups present. Each spectrum was obtained with a resolution of 4 cm^{-1} and consisted of 128 scans per sample. Infrared spectroscopy enabled the detection and characterization of chemical functional groups within the cellulose samples.

1.3.2 Scanning Electron Microscope (SEM)

The morphology of both rice straw and the extracted cellulose samples was analyzed using scanning electron microscopy (SEM) with a HITACHI SU8220 instrument equipped with OXFORD Instruments. For sample preparation, the samples were dispersed in 70% (v/v) ethanol and deposited onto a metal stub covered with aluminum tape. After drying with pressurized air, a 12-15 nm platinum layer was sputter-coated onto the samples using a mini sputter coater (SC7260, Quorum Technologies) under vacuum conditions. The samples were then examined under the microscope to observe changes in morphology and fibrillar structure resulting from the treatment processes.

1.3.3 X-Ray Diffraction Analysis (XRD)

To determine the crystallinity, X-ray diffraction was performed on the raw rice straw and the extracted cellulose samples using the Bruker D2 (Billerica, MA, USA) with Cu-Kα radiation (⅄ = 1.5406 Å), continuous scan (2θ) at 30 mA and 40 kV. Initially, the dried sample was positioned on a 0.5mm glass sample holder and evenly spread using another glass slide to create a smear. Subsequently, the sample was scanned over an angular range from 5° to 50°, with a scanning rate set at 0.02°per step increment. The change in the degree of crystallinity of cellulose samples was expressed in terms of the crystallinity index (*CrI)*. The *CrI* value was determined based using the Segal method, which relates the height of the crystalline peak corresponding to the (002) lattice place (I_{002}) to the intensity of the amorphous region (I_{am}), and were used to solve for the CrI as shown in **Equation (2)** (Mains 2021).

$$\text{CrI}\ (\%) = \frac{(I_{002} - I_{am})}{I_{002}} \times 100\% \quad (2)$$

Where $I_{002}$is the maximum diffraction intensity, located around 2θ = 22.5° and corresponds to the crystalline region; I_{am} is the minimum diffraction intensity, located around 2θ = 18° and corresponds to the amorphous material.
Following that, the crystallite size (D) of both raw rice straw and extracted cellulose samples was determined using Scherrer's equation, which is presented in **Equation (3)** (ISMAIL et. al, 2020).

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (3)$$

Where θ is the Bragg's angle in degrees, β is the full width at half maximum (FWHM) intensity of the peak at diffraction plane 002 in radian, ⅄ is 0.15406nm (the wavelength of X-ray diffraction equipment), and K = 0.91 (constant).

2 Results and Discussion

2.1 Effect of alkali concentration during delignification

Table 2 presents the cellulose yield obtained using different concentrations of alkali. The yield varied from 29.4% to 10.7% as the alkali concentration increased from 3% to 11%. This inverse relationship can be attributed to the increase in sodium hydroxide (NaOH) concentration, which leads to a higher amount of alkaline metal hydration ions. These ions accelerate the alkaline degradation of cellulose (Li et. al, 2017), ultimately reducing the cellulose yield.

Table 2. Cellulose yield at different alkali concentrations.

Run	Alkali concentration (%)	Temperature (°C)	Time (min)	Cellulose Yield (%)
1	3	60	60	29.4
2	7			22.8
3	11			10.7

When compared to existing literature, the maximum cellulose yield in this study (29.4%) closely aligns with previously reported yields ranging from 29% to 55% (Sharma et. al, 2023). This suggests that the eco-friendly extraction method employed here successfully isolates cellulose from rice straw at yields comparable to other methods. While the study demonstrates a clear relationship between alkali concentration and cellulose yield, future research could explore additional factors or optimizations to further enhance the yield.

2.2 Characterization of Cellulose

The resultant white products obtained after chemical treatment for several conditions of rice straw, were confirmed to be cellulose by spectra of Fourier Transform Infrared Spectroscopy (FTIR). Meanwhile, for the micrographs of Scanning Electron Microscopy (SEM), the morphology and the appearance of the cellulose can be observed, while X-Ray Diffraction (XRD) analysis was used to identify the characteristics and crystallinity of the resultant white samples.

2.2.1 Sample Functional Groups

The characterization of the extracted cellulose fibers involved FTIR analysis to identify the functional groups present in untreated rice straw and rice straw treated with different concentrations as presented in **Figure 1** below. All the spectra were quite similar, indicating that a similar structure predominated in all the samples.

The broad band centred at 3411 cm^{-1} is attributed to the O─Hgroup stretching vibrations in cellulose, while the peak around 2904 cm^{-1} corresponds to the symmetric and asymmetric C─H group stretching, indicating the presence of lignin (Nazir et. al, 2013). The intensity of the O─H and C─H stretching vibration peaks in the treated rice straw samples with higher alkali concentration is lower than in the treated fibres with lower alkali concentration. This decrease is significant because it highlights the removal of amorphous components, such as hemicellulose and lignin, during the alkali treatment. Additionally, the bleaching process involving hydrogen peroxide may contribute to the reduction in cellulose and lignin content.

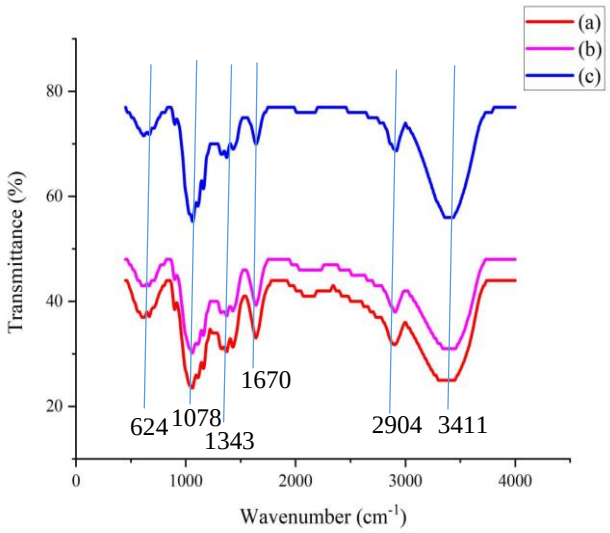

Fig. 1 FTIR spectra of extracted cellulose using11%; (b) 7%; and (c) 3% alkali concentrations.

A small band at 1670 cm^{-1} was strongly associated with the C=O stretching mode of the lignin aromatic ring. A reduction in its intensity as the alkali concentration increases was related to modification of the aromatic ring and corresponds to delignification (Martelli-Tosi et. al, 2017). Similarly, the absorbance peaks (1343 cm^{-1} and 624 cm^{-1}), which correspond to the stretching of the aromatic C─H bonds and hydroxyl groups (─OH) respectively, are characteristic of lignin group. These peaks exhibit a decrease in intensity as the alkali concentration increases. This confirms the successful removal of lignin from the rice straw. Additionally, the intensity of the band at 1078 cm^{-1}, which is attributed to C─O asymmetric stretching, decreases after alkali treatment. This characteristic peak is associated with the hemicellulose group, and its reduction confirms the removal of hemicellulose, thereby increasing the cellulose content. The decreasing intensity and transmittance percentage serve as indicators of the reduced content of lignin, hemicellulose, and other impurities in the cellulose fibers.

2.2.2 Morphological Features of Extracted Samples

The surface morphology of the extracted cellulose was characterized via scanning electron microscope (SEM). **Figure 2** below illustrates the extracted cellulose under different conditions. As depicted in Figure 2, the isolated fibrils that are well-separated from each other, indicating the complete removal of hemicelluloses, lignin, waxes, etc. The average diameter of fiber bundles decreased with increasing alkali concentration, measuring (18.3 ± 5.3) µm, (5.9 ± 1.1) µm, and (5.0 ± 0.7) µm at concentrations of 3%, 7%, and 11%, respectively. This trend is illustrated in the bar chart presented in **Figure 3** below, showing a decrease in fiber size indicative of the effective removal of lignin by alkaline treatment. The external surface exhibited a smoother appearance, likely due to the removal of inorganic particles such as silica and metal components (Nazir et. al, 2013).

To investigate the surface morphological changes of the fibers, SEM analysis was conducted at 1000× magnification, as shown in Figure 2(ii). The SEM micrograph of cellulose treated using 3% alkali concentration reveals a rough surface with dense deposits of lignin, hemicellulose, ash, and other spherical structures. However, as the alkali concentration increases, the surface becomes progressively smoother. This smoothing is due to the effective removal of amorphous lignin and hemicellulose, consistent with findings by Chen et. al, (2013) (Chen et. al, 2013). At higher alkali concentrations, the SEM images (Fig. 2 (b), and (c)) show significant delinking of carbon bonds, leading to loosely packed fiber bundles and a reduction in amorphous components. This results in smoother and more structurally refined cellulose fibers, highlighting the efficacy of the alkali treatment.

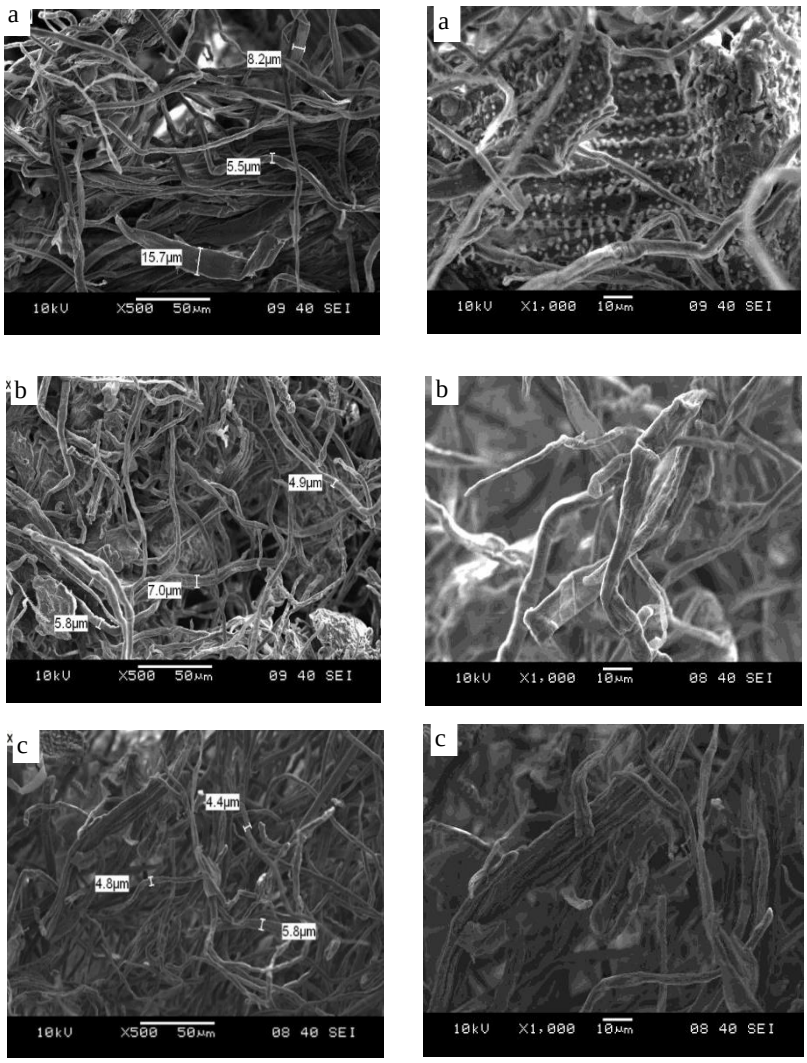

Fig. 2 SEM micrographs of extracted fibers using (a) 3%; (b) 7%; and (c) 11% alkali concentration at the magnification of (a) 500× and (b) 1000×

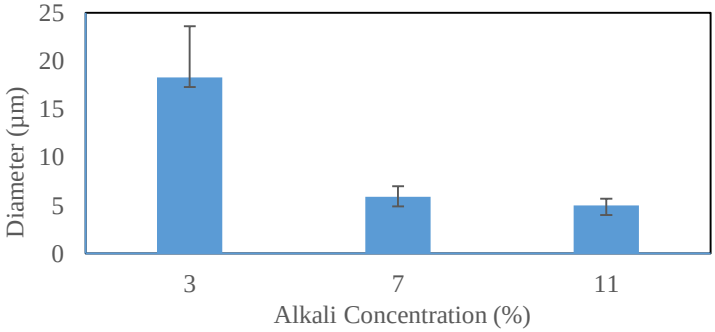

Fig 3. Bar chart of diameter of fibers at different alkali concentrations.

2.2.3 Sample Crystallinity

In this study, XRD patterns were analyzed to understand the effects of different treatments on the crystalline structure of rice straw samples. **Figure 4** presents the XRD diffractogram for treating samples at different conditions, showing prominent peaks at 16.24°, 22.47°, and 34.99° of 2θ values. Two distinct characteristic

peaks are observed at 2θ = 16.24° and 2θ = 22.47°, representing the amorphous and crystalline structures, respectively. These patterns are typical of semicrystalline cellulosic materials, featuring a broad amorphous band and a well-defined crystalline peak. The presence of lignin and hemicelluloses contributes to the amorphous structure in both untreated and treated samples, while the crystalline structure predominantly arises from cellulose.

In all analyzed samples, the diffractogram profile indicated that cellulose type I predominated, with the main diffraction intensity at about 2θ = 22.46° (plane 002) and identifiable peaks at 2θ = 16.24° (plane 101) and 2θ = 34.99° (plane 004). This observation aligns with the findings of Martelli-Tosi et. al, (2017) (Martelli-Tosi et. al, 2017).

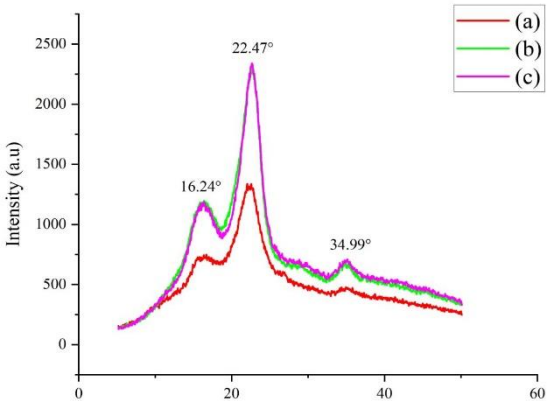

Fig 4. . X-Ray Diffraction spectra for treated rice straws at (a) 3%; (b) 7%; and (c) 11% alkali concentration..

As the treatment concentration increases from 3% to 11%, there is a noticeable enhancement in crystallinity, evidenced by the increasing intensity and definition of these peaks. Additionally, the peak at 34.99° indicates improved crystalline order in the samples treated. These observations align with the FTIR and SEM findings, confirming the effective removal of amorphous hemicelluloses and lignin during the cellulose extraction process from rice straw.

Table 3 below presents the Crystallinity Index (*CI*) and the crystallite size for each sample. The calculated peak intensities demonstrate a clear trend of increasing crystalline with higher treatment concentrations, supporting the observation of an enhanced crystalline structure. The results presented in Table 3 highlight the impact of alkali concentration on the CI and crystallite size of the extracted cellulose. The *CI* increased consistently from 47.85% to 51.37% as the alkali concentration rose from 3% to 11%. This trend indicates the progressive removal of amorphous components, such as lignin and hemicellulose, with higher alkali concentrations. The increase in *CI* suggests enhanced delignification and structural ordering of the cellulose fibers.

Conversely, the crystallite size exhibited a non-linear trend, decreasing from 1.97 nm at 3% alkali concentration to 1.32 nm at 7%, followed by an increase to 1.76nm at 11%. This behavior can be attributed to the interplay between cellulose degradation and recrystallization during alkali treatment. At moderate alkali concentrations (7%), the degradation of cellulose chains may dominate, leading to a reduction in crystallite size. At higher concentrations (11%), partial recrystallization or aggregation of cellulose molecules may occur, resulting in an increase in crystallite size. These observations underscore the complexity of the delignification process, where the alkali concentration not only influences the removal of impurities but also affects the structural properties of the extracted cellulose. Compared the values in Table 3 to findings reported by Shahid Nazir et. al, (2013) (Nazir et. al, 2013) and Al-Rajabi & Haan (2022) (Al-Rajabi and Haan 2022), the *CI* and crystallite size of raw fibers were calculated as 43.9% and 31.5 nm, respectively. In contrast, for the extracted cellulose in this study, CI was measured at 70% with a crystallite size of 9.8 nm.

The difference between the *CI* and crystallite size values could be attributed to differences in methodologies and the inherent variability in the raw fiber samples. Nevertheless, the results demonstrate that the *CI* increases following the series of treatment processes. Furthermore, the decrease in crystallite size confirms the degradation of the amorphous structure, leading to the transformation into a structure with higher crystallinity.

Table 3. The CI and crystallite size of treated rice straws at different conditions.

Alkali concentration (%)	CI (%)	Crystallite size, D (nm)
3%	47.85	1.97
7%	48.33	1.32
11%	51.37	1.76

Coclusion

This study successfully developed an environmentally friendly method extracting pure cellulose from rice straw. The effect of the alkali concentration during delignification for cellulose extraction from rice straw was conducted. The study investigated the effects of alkali concentration on cellulose yield and color index. FTIR studies confirmed the removal of hemicelluloses, lignin, and other inert materials. SEM observations indicated that the extracted cellulose exhibited a fibrillar structure with decreasing diameter as the alkali concentration increased. X-ray diffraction (XRD) analysis confirmed the crystalline nature of the extracted cellulose. The degree of cellulose crystallinity increases with alkali concentration as the amorphous constituents were removed during the treatments. The average CI was increased from 47.85% for treated fibers using low alkali concentration to 51.37% for treated fibres using high concentration. However, a trade-off between yield and purity was observed. While lower alkali concentrations were more environmentally friendly and yielded higher amounts of cellulose, FTIR, SEM, and XRD results revealed that these samples contained residual impurities, indicating lower purity. This highlights the need to balance cellulose yield with purity when optimizing alkali concentrations for cellulose extraction. In overall, this study demonstrates the effectiveness of alkaline treatment in extracting high-quality cellulose from rice straw, contributing to sustainable agricultural waste management and the production of valuable cellulose.

Acknowledgement

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Nomenclature

D	=	crystallite size	[nm]
I_{002}	=	the maximum diffraction intensity	[a.u.]
I_{am}	=	the minimum diffraction intensity	[a.u.]
m_0	=	original weight before delignification of the cellulose fibers	[g]
m'	=	final dry weight of the cellulose fibers	[g]
λ	=	wavelength of X-ray diffraction equipment	[nm]
β	=	full width at half maximum intensity of the peak at diffraction plane 002	[radian]
θ	=	Bragg's angle	[°]

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