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Isolation and Characterization of Cellulose from Biomass: A Methodological Review

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ABSTRACT: *The isolation and characterization of cellulose from biomass have garnered increasing research interest due to cellulose's pivotal role in supporting sustainable material innovations across various industries. This study presents a comprehensive review of recent advancements in cellulose isolation methods, including chemical, mechanical, and enzymatic techniques, emphasizing their efficiencies in yield and purity. A bibliometric analysis using VOSviewer was also conducted to map research trends, identify leading authors and institutions, and visualize keyword co-occurrence networks from publications between 2015 and early 2025. The bibliometric results revealed three major thematic clusters in cellulose research: extraction and pretreatment technologies, material characterization techniques, and structural property analysis. Publication trends indicated dynamic research developments, particularly a surge in interest following advances in green extraction technologies. Overall, this review highlights the shift towards sustainable cellulose isolation strategies, evaluating the advantages and disadvantages of various methodologies. It provides insights into the strengths and limitations of current techniques, while also outlining potential future directions for research and industrial applications.*

Keywords:*Biomass, Bibliometric Analysis, Cellulose, Characterization, Isolation.*

1. INTRODUCTION

The increasing demand for sustainable materials has driven significant interest in cellulose, the most abundant natural polymer on Earth (T. Li et al., 2021; Shaikh et al., 2021). As industries seek eco-friendly alternatives to synthetic polymers, cellulose stands out due to its wide availability and compatibility with green technologies (Muthamma & Sunil, 2022). Its role as a fundamental structural component in plant cell walls highlights its importance in nature and its potential value in various industrial applications (Rongpipi et al., 2019).

Cellulose is primarily obtained from biomass sources such as agricultural residues, forestry waste, and industrial by-products. Agricultural residues such as corn stalks, rice husk, sugarcane bagasse and banana peels are commonly used due to their high cellulose content and widespread availability (Gapsari et al., 2023; Zheng et al., 2019). Forestry waste, including wood chips, sawdust and baek, provides relatively pure and long chain cellulose (Sutrisno et al., 2020). Meanwhile, industrial by-products from the paper, food, or palm oil industries, such as sludge, pulp waste, and empty fruit bunch (Amrillah et al., 2023; Devita & Srimurni, 2022; Kholis et al., 2019). These renewable resources make cellulose a highly attractive material for supporting a circular economy (T. Li et al., 2021). Beyond its abundance, cellulose possesses remarkable properties including biodegradability, renewability, and high mechanical strength, making it suitable for applications ranging from bioplastics and textiles to pharmaceuticals and advanced composites (Reid et al., 2017; Weng et al., 2021).

Biomass is a complex structure composed of cellulose intertwined with non-cellulosic components such as lignin, hemicellulose, and various extractives. These components form a rigid and protective matrix around cellulose, making direct access and utilization challenging (Dube et al., 2023). Therefore, to obtain pure cellulose, it is essential to selectively remove these associated substances without damaging the cellulose fibers (Muthukumar & Chidambaram, 2023).

To achieve effective isolation, several methods can be employed, including chemical, mechanical, and enzymatic treatments. Chemical methods often involve the use of alkaline or acidic solutions to break down lignin and hemicellulose (Hozman-Manrique et al., 2023). Mechanical techniques, such as mechanical grinding or steam explosion, help disrupt the biomass structure, making chemical treatments more effective (Dou et al., 2016). Enzymatic processes offer a more environmentally friendly approach by using specific enzymes to degrade non-cellulosic materials selectively (Pimentel et al., 2021). The selection of the method depends on the type of biomass, the desired purity of cellulose, and the intended application.

The increasing demand for sustainable and eco-friendly materials has driven extensive research on cellulose extraction and purification from diverse biomass sources. A comprehensive understanding of the strengths and limitation of different isolation techniques is essential for selecting appropriate methods based on specific applications. This study aims to evaluate the advantages and disadvantages of various cellulose isolation methods, providing a foundation for selecting the most appropriate method based on specific goals and needs. Additionally, the research offers insights into developing combined approaches by integrating multiple isolation methods to achieve more efficient and optimal results in cellulose purification, both for research and industrial applications.

2. METHODOLOGY

2.1. Data Collection

To systematically examine the collected data, the Methodi Ordinatio approach was utilized, relying solely on sources from the Scopus database (Pagani et al., 2015). Data gathering took place on April 27, 2025, using the search terms: “Cellulose OR Cellulosic OR Cellulose fiber OR Cellulose polymer” combined with “Isolation OR Extraction OR Process OR Approach,” “Chemical OR Enzymatic OR Mechanical OR Biological,” and “Yield OR Efficiency OR Quality OR Characterization,” targeting publications from 2015 to the end of April 2025. A total of 384 documents were retrieved. After sorting the data using Mendeley to check for duplicate records, it was confirmed that no duplicates existed, maintaining the final dataset at 384 documents. Table 1 presents the principal stages undertaken in the data analysis methodology of this study.

Following this, a content analysis was conducted focusing on several dimensions, including document type, keyword usage, publication year, and journal citation patterns.

2.2. Bibliometric Analysis and Mapping Publications

Bibliometric analysis was conducted utilizing VOSviewer version 1.6.18 for Windows, following established protocols as documented in prior research. The metadata examined encompassed elements such as authorship, co-authorship, titles, abstracts, and keywords. To ensure analytical rigor, a minimum occurrence threshold of five was applied to each term, and duplicate or non-relevant keywords were systematically excluded to uphold data quality and relevance. The refined dataset was subsequently processed in VOSviewer, facilitating the visualization and assessment of emerging research patterns through bibliometric mapping. Furthermore, the graphical representation of findings using DataWrapper contributed to enhanced clarity and interpretability of the results.

Table 1. Main steps to conduct the literature review.

Steps		Descriptions	
Database search	I	Initial portofolio	
		Database	Scopus
		Keyword	>100
		Numbers of documents	384
Filtering procedures	II	Elimination of duplicates and limiting to recent 11 years publication (from 2015 to 2025)	-
	III	Screening title and keywords	
	IV	Reading of abstract	
	V	Reading of full text	
		Final Portofolio	
		Number of documents	384
		Keyword	50
Content analysis	VI	Type of documents	
		Keywords	
		Year of publication	2015-2025
		Journal and Citation	

3. CHARACTERISTIC OF RELATED LITERATURES

3.1. Type of Document

A total of 384 documents were obtained from the Scopus database during the data collection on April 27, 2025, encompassing publications from 2015 up to the end of April 2025. **Figure 1.** displays the distribution of documents were categorized based on their type. Revealing that the majority consisted of research articles, accounting for 341 documents. Additionally, 30 documents were classified as review papers, which provide comprehensive overviews and analyses of existing research on cellulose isolation and characterization.

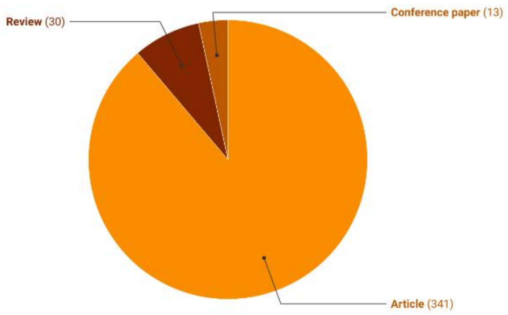

Figure 1. Distribution of the number of documents regarding types of published documents.

Meanwhile, 13 documents were identified as conference papers, representing preliminary findings or ongoing research presented at conferences. This distribution indicates a strong focus on original research in the field, complemented by review articles that synthesize knowledge and a small portion of conference contributions reflecting emerging studies.

3.2. Keywords

An additional analysis was conducted on the keywords consolidated in the final portfolio through bibliometric methods to develop a visual network map. Keywords taken from published articles are used to describe the research landscape and to help identify major topics and trends in the field. The bibliometric analysis was based on the co-occurrence of keywords appearing in more than two publications. For this study, 50 keywords associated with the development of isolation and characterization were selected.

This study includes 50 keywords that were carefully selected based on their relevance to cellulose research. The outcomes of the bibliometric study are illustrated in Figure 2, which presents a visual network map generated through VOSviewer, effectively highlighting the relationships and co-occurrence patterns among the selected terms.In the map, the keywords were automatically grouped into three major clusters distinguished by color coding: the red cluster, the green cluster, and the blue cluster.

Each cluster highlights distinct thematic focuses within the field of cellulose research (Figure 3). The green cluster emphasizes the extraction, pretreatment, and biochemical conversion of biomass, involving keywords like “biomass”, “lignocellulose”, “hydrolysis” and “fermentation”. The red cluster primarily focuses on material properties and characterization techniques, including terms such as “cellulose”, “chemical structure”, “morphology”, “scanning electron microscopy”, “fourier transform infrared”, and “x ray diffraction”. The blue cluster is smaller and focuses on crystallinity and structural characterization, highlighting keywords such as “crystallinity”, “degradation” and “characterization”.

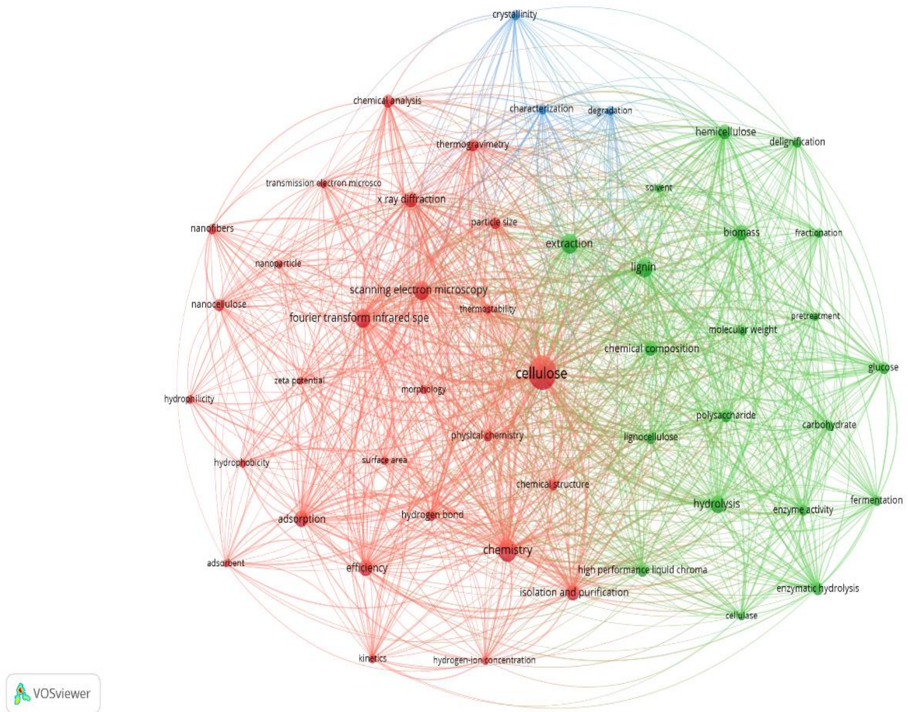

Figure 2. A visual map network of keyword co-occurences derived from the references supporting the development of this study. Created with: VOSviewer

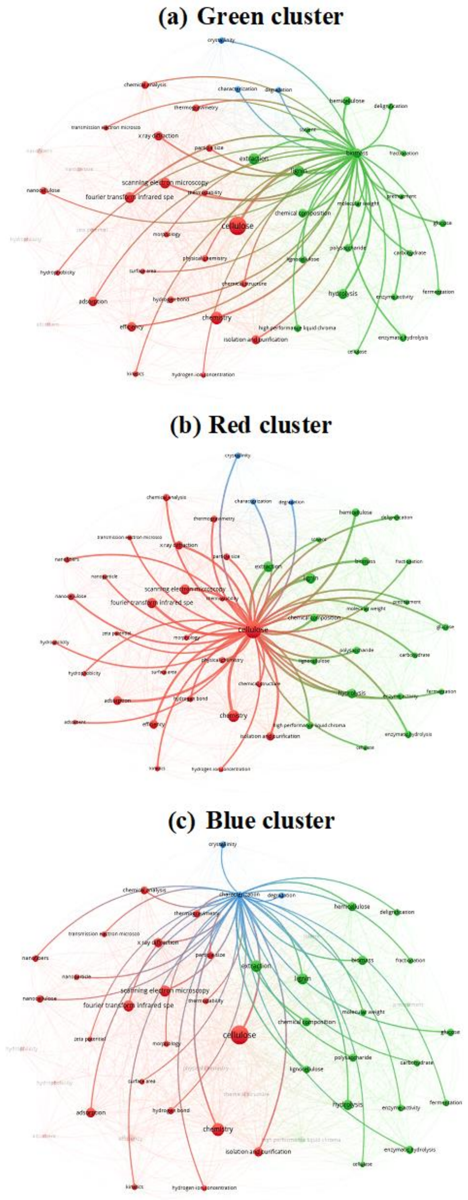

Figure 3. A visual map based each cluster (a) Green cluster, (b) Red cluster and (c) Blue cluster.

These clusters suggest broad directions for future research, such as the advancement of analytical methodologies, optimization of cellulose extraction and conversion processes, and the exploration of structure–property relationships. The network visualization thus offers valuable insights into the intellectual structure and emerging themes within the field of cellulose-related studies.

3.3. Year of Publication

The publication trend from 2015 to early 2025 (Figure 4) demonstrates fluctuating research interest in cellulose isolation and processing. From 2015 to 2017, the number of studies increased steadily from around 20 to 30 per year. In 2018 and 2019, there was a slight decline, but the numbers stayed stable. A sharp rise occurred in 2020 and 2021, reaching 50 to 55 publications. This was followed by a drop in 2022 and 2023, possibly due to shifting research priorities. In 2024, the number peaked at 65, likely because of new isolation methods and growing industrial applications. The 2025 data appear lower, but only cover the period up to April. Overall, the trend shows steady growth in research, supported by technological advances and industrial interest.

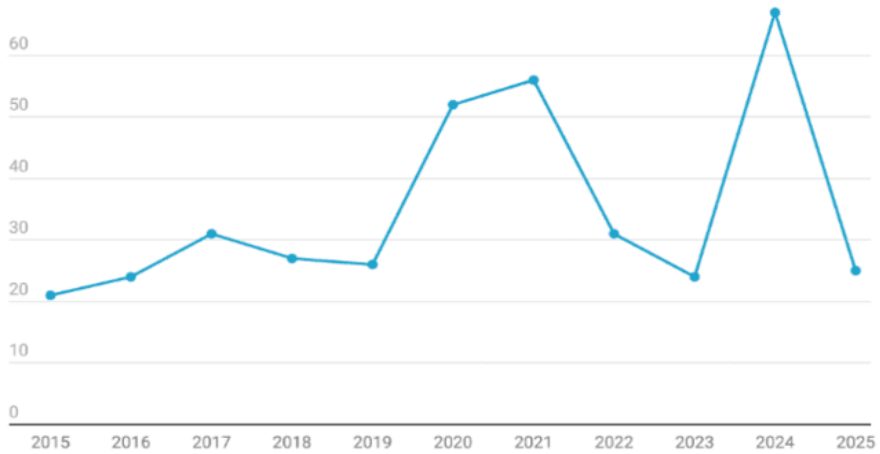

Figure 4. Distribution of the number of published documents over the period from 2015 to 2025 (data retrieved per April 27, 2025). Created with: DataWrapped.

3.4. Most Documents Published on Journal and Most Cited Document

Table 2. a list journals based on the number of publications, their highest quartile ranking, total citations and the most cited papers. The *International Journal of Biological Macromolecules* leads with 59 relevant documents, categorized in the Q1 quartile, and accumulating a total of 1,300 citations. Following it is *Bioresource Technology*, with 40 publications, also in Q1, but notably achieving the highest total citations among all, reaching 2,139 citations, with a key paper by Karimi and Taherzadeh in 2016. Subsequently, *Industrial Crops and Products* contributed 19 documents, *Chemosphere* added 15 papers, and *Carbohydrate Polymers* published 11 relevant articles, all consistently classified as Q1 journals. These results highlight that research on cellulose isolation and characterization is being published in high-impact journals, mainly within the fields of biological macromolecules, bioresources, industrial applications, and environmental sciences, reflecting its multidisciplinary importance.

Table 2. Journal with greatest number of publications addressing the topic of "Isolation and Characterization Cellulose"

Journal Name	Number of Relevant Documents	Highest Quartile*)	Total Cites	Most Cited
International Journal Of Biological Macromolecules	59	Q1	1.300	(Trache et al., 2016)
Bioresource Technology	40	Q1	2.139	(Karimi & Taherzadeh, 2016)
Industrial Crops and Products	19	Q1	604	(Kouadri & Satha, 2018)
Chemosphere	15	Q1	831	(Nie et al., 2018)
Carbohydrate Polymers	11	Q1	935	(Julie Chandra et al., 2016)

*) Based on data retrieved from Scimago Journal &16 Country Rank on April 27, 2024.

4. RECENT DEVELOPMENT ON DIFFERENT METHODS OF CELLULOSE ISOLATION

Recent advancements in the extraction of cellulose from biomass have been propelled by the growing demand for sustainable and renewable resources. A variety of chemical, mechanical and enzymatic methods have been developed and optimized to improve extraction processes (Manzano et al., 2025). Each approach offers distinct advantages and challenges, depending on the nature of the biomass and the intended application of the cellulose.

Chemical methods, such as alkali treatment, acid hydrolysis, and organosolv processes, are widely employed to remove non-cellulosic components like lignin and hemicellulose (Birhanu et al., 2024). Although these approaches can achieve high levels of cellulose purity, they often involve harsh reagents, leading to environmental concerns and increased operational costs. Physical methods, including high-pressure homogenization and ultrasonication, are increasingly utilized to aid in cellulose isolation, either as standalone techniques or in combination with chemical or enzymatic treatments to enhance extraction efficiency (Manzano et al., 2025). Meanwhile, enzymatic techniques provide a greener alternative by employing specific enzymes to selectively degrade lignin and hemicellulose, minimizing chemical consumption and environmental impact, although these methods typically require longer processing times and precise optimization (Yang & Chen, 2023).

Recent developments in cellulose isolation techniques focus on enhancing yield and purity while maintaining alignment with green chemistry. These advancements aim to minimize environmental impact by reducing the consumption of energy, water, and hazardous chemicals during the isolation process. These advancements support the establishment of environmentally sustainable approaches to cellulose production, aligning with the broader transition toward a circular bioeconomy.

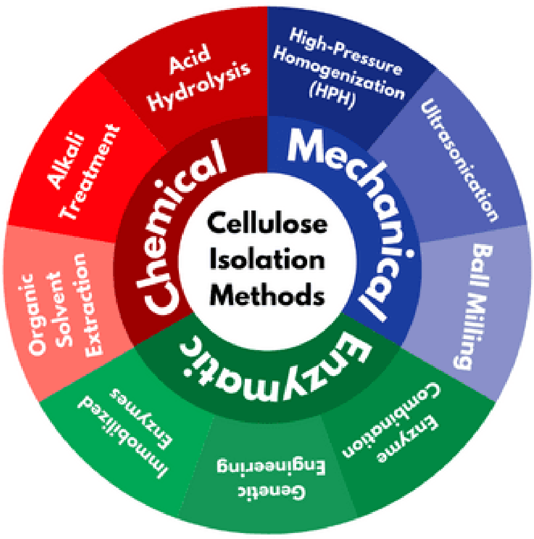

Figure 5. Cellulose Isolation Methods

4.1 Chemical Methods

Chemical methods for isolating cellulose involve the use of acids, alkalis and organic solvents to selectively degrade lignin and hemicellulose while preserving the cellulose structure (Birhanu et al., 2024). These methods are widely used due to their efficiency in producing high-purity cellulose from various biomass resources. However, their environmental impact and potential degradation of cellulose are key concern. Recent developments in this area include:

4.1.1 Acid Hydrolysis

Acid hydrolysis is a commonly used method to break down hemicellulose and disrupt the lignin structure, facilitating cellulose isolation (Birhanu et al., 2024). Acid hydrolysis utilizes strong acids such as sulfuric acid (H_2SO_4) and hydrochloric acid (HCl) and weak acids such as acetic acid, formic acid and oxalic acid to dissolve hemicellulose and lignin, leaving behind purified cellulose (Weerasooriya et al., 2020). This process involves breaking the glycosidic bonds in hemicellulose removal and relatively short reaction times. The advantages of acid hydrolysis include high efficiency in lignin and hemicellulose removal and relatively short reaction times (Lu et al., 2021). Birhanu et al., (2024) studied the invasive weed Senna didymobotrya and successfully isolated cellulose with a yield of 37.5% using a combination of sulfuric acid, sodium chlorite, and potassium hydroxide treatments. In the study conducted by Pratama, Mahardika et al. (2024), microcrystalline cellulose (MCC) was produced from belulang grass (Eleusine indica) using diluted hydrochloric acid, followed by washing and neutralization, resulting in a cellulose yield of 74.89%. Strong acids can degrade cellulose, resulting in reduced yields and the formation of by-product such as furfural and hydroxymethylfurfural (HMF). Altough these compounds have potential economic value, they are considered undesirable in this context because their presence indicates cellulose breakdown, which undermines the goal of maintaining the structural integrity needed for efficient regeneration and functionalization (Duan et al., 2024).

To address this issue, researchers have explored weaker organic acids such as acetic acid, formic acid, and lactic acid. These acids are effective at breaking down hemicellulose while reducing cellulose degradation. For instance, Bao et al., (2022) demonstrated that using tropic acid to pretreat eucalyptus resulted in an 85.78% yield of hemicellulose-derived sugars, while minimizing xylose degradation compared to conventional acidic methods. This approach not only improved hemicellulose separation but also maintained the high crystallinity of the remaining cellulose, essential for producing high-purity cellulose.

In recent years, advancements in acid hydrolysis have focused on combining chemical treatments with physical enhancement techniques. Ratnakumar et al., (2022) demonstrated that using chemo-ultrasonic methods on rice straw significantly increased cellulose yield. The study reported that smaller particle sizes yielded higher cellulose extraction rates, with particles under 75 µm producing a cellulose yield of 27.19%, while larger particles (150 to 250 µm) yielded 38.31%. Moreover, ultrasonic-assisted extraction improved the cellulose nanofiber (CNF) yield to 64% from smaller particles, indicating the effectiveness of combining chemical and ultrasonic methods.

Integrating acid hydrolysis with physical treatments like ultrasonic and microwave methods significantly improves cellulose yield and quality. Using weaker organic acids instead of strong mineral acids reduces cellulose degradation, resulting in higher purity. These advancements offer a sustainable approach to cellulose isolation from both agricultural residues and invasive plants, supporting the development of high-quality cellulose for industrial applications.

4.1.2 Alkali Treatment

Alkali treatment primarily involves the use of sodium hydroxide (NaOH) or potassium hydroxide (KOH) to break down lignin and partially remove hemicellulose. Alkaline treatment works by disrupting ester and ether linkages in lignin, leading to its solubilization. It also causes cellulose fibers to swell, increasing their reactivity (Marda S.R et al., 2023). The primary benefits of this methods include selective lignin removal and enhanced cellulose accessibility (J. Li et al., 2021). Several studies have investigated the optimization of alkali treatment for cellulose extraction. Pratama et al. (2024) utilized a combination of acid hydrolysis and TEMPO-mediated oxidation to isolate cellulose nanofibrils (CNF) from various biomass sources, such as corn cob, bagasse, and waste wood. The cellulose yields ranged from 25% to 34%, with the highest yield obtained from corn cob. Luo et al. (2020) utilized a 5% NaOH solution in combination with alcohol-mediated precipitation to extract cellulose from viscose fiber plants, achieving a cellulose yield of 70.5%. Rieland & Love (2020) integrated ionic liquids with a 6% NaOH treatment to recover cellulose from agricultural biomass, obtaining a yield of 65.8%. Furthermore, Cai et al. (2020) reported that using a 3% NaOH solution for producing cellulose nanofibers, followed by hydrogel formation, achieved a yield of 72.1%.

However, challenges include high water consumption, chemical recovery issues, and potential partial degradation of hemicellulose. Recent advancements focus on optimizing alkali concentrations, reaction times, and incorporating oxidation agents such as hydrogen peroxide (H_2O_2) to improve lignin removal while reducing environmental impact (Owolabi et al., 2017). Additionally, novel deep eutectic solvents (DES) have been investigated as greener alternatives to traditional alkaline treatments (Zhang et al., 2024).

4.1.3 Organic Solvent Extraction

The use of organic solvents in cellulose isolation has garnered substantial interest owing to its promising potential to selectively dissolve lignin while preserving cellulose. Ionic liquids (ILs) and deep eutectic solvents (DES) have gained prominence as viable substitutes for traditional solvents. These solvents offer significant advantages, including recyclability, environmental compatibility, and high efficiency in cellulose extraction, although challenges related to cost and large-scale application persist (Norfarhana et al., 2024; Zhang et al., 2024). ILs, such as imidazolium-based and phosphonium-based salts, can efficiently disrupt lignin structures, facilitating cellulose separation. Unlike conventional chemical treatments, ILs can be recycled, making them more environmentally friendly. Rieland & Love (2020) explored the use of ILs for cellulose extraction from biomass. ILs, particularly those based on imidazolium and phosphonium salts, have shown excellent capacity to disrupt lignin structures, thereby facilitating cellulose isolation. The cellulose solubility achieved through ILs ranged from 10–20 wt%, indicating their efficiency in cellulose recovery. However, their high cost and recyclability challenges hinder large-scale applications (Rieland & Love, 2020).

DES, composed of biodegradable and low-toxicity compounds such as choline chloride and urea, have been explored for lignin removal due to their low environmental impact and ease of preparation. Zhang et al. (2024), a DES composed of glycerol and choline chloride was used for the pretreatment of poplar wood. The solid cellulose product obtained after pretreatment exhibited a purity level exceeding 80%. This finding highlights the potential of DES systems in achieving high cellulose purity while maintaining the structural integrity necessary for further applications. Recent studies have focused on optimizing IL and DES compositions to maximize efficiency while minimizing cost and toxicity.

4.2 Mechanical Methods

Mechanical methods involve the use of physical forces to break down biomass and separate cellulose fibers without extensive chemical treatment. These methods are attractive due to their scalability, environmental friendliness, and ability to preserve cellulose integrity. The three primary mechanical methods for cellulose isolation are High-Pressure Homogenization (HPH), Ultrasonication, and Ball Milling (Amiralian et al., 2015).

4.2.1 High-Pressure Homogenization (HPH)

High-Pressure Homogenization is a technique that uses high shear forces to break down biomass and liberate cellulose fibers. This method involves forcing biomass suspensions through a narrow valve or nozzle under extremely high pressure (typically 50–300 MPa). The repeated application of shear, cavitation, and impact forces disrupts the lignocellulosic matrix, facilitating cellulose separation (Samsalee et al., 2023). Increasing the number of HPH cycles significantly affected the particle size, crystallinity index, and thermal stability of cellulose nanofibrils. The crystallinity index increased with the number of HPH cycles, indicating a more ordered crystalline structure (Samsalee et al., 2023). HPH increased the cellulose extraction efficiency by up to 21% when applied before chemical hydrolysis, compared to an increase of about 6% when applied afterward. The resulting cellulose showed a finer and defibrillated fiber morphology, with an average particle size of about 315 µm, compared to 630 µm in the sample without HPH (Pirozzi et al., 2022).

4.2.2 Ultrasonication

Ultrasonication involves the use of high-frequency ultrasonic waves (typically 20–100 kHz) to break down lignocellulosic structures. The ultrasonic energy generates acoustic cavitation—small bubbles that rapidly collapse, releasing localized high energy. This process effectively disrupts the biomass structure, loosening the bonds between lignin, hemi cellulose, and cellulose (Hoo et al., 2022). The use of ultrasonic methods in cellulose isolation can reduce the hydrophilicity of cellulose fibers (Malik et al., 2023). Ultrasonic isolation of cellulose provides additional mechanical disturbance which increases the furity of cellulose to a greater extent reaching 65.3% (Mohan et al., 2024). while in another method using a combination of chemical-ultrasonication treatment produces a high yield of cellulose nanofibers (89.35%) (Syafria et al., 2018)

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4.2.3 Ball Milling

Ball milling is a mechanical grinding process that reduces biomass particle size using rotating cylindrical chambers filled with steel or ceramic balls. The repeated impact, friction, and shear forces disrupt the crystalline structure of biomass, increasing cellulose exposure and reducing particle size to micro- or nanoscale levels (Chen et al., 2022). factors that must be considered when using milling-ball are milling-ball size, milling time and ball-to-cellulose mass ratio (Zhang et al., 2015). Currently, ball milling is widely used for cellulose preparation due to its simple operation, relatively inexpensive equipment usage, and wide application to most types of biomass. Dynamic light scattering tests showed that cellulose using the milling method produced sizes of 240 ± 12 nm and 10 μm(Rahimi Kord Sofla et al., 2016). A chemical-mechanical process involving ball milling combined with acid hydrolysis produces yields of up to 81% (Souza et al., 2025).

4.3 Enzymatic Methods

Enzymatic isolation of cellulose is a sustainable and highly specific method that utilizes cellulases and other hydrolytic enzymes to selectively degrade non-cellulosic constituents, including lignin and hemicellulose, while preserving the cellulose structure. Unlike chemical or mechanical methods, enzymatic processes operate under mild conditions, reducing energy consumption and minimizing the production of harmful byproducts. This approach has attracted considerable interest owing to its ecological advantages and efficiency in obtaining high-purity cellulose (Yang & Chen, 2023).

4.3.1 Enzyme Combinations

One of the key advancements in enzymatic cellulose isolation is the strategic blending of different cellulases and hemicellulases to maximize efficiency. Cellulases, such as endoglucanases, exoglucanases, and β-glucosidases, collaboratively degrade cellulose fibers into smaller sugar molecules through a coordinated enzymatic process. Hemicellulases, including xylanases and mannanases, contribute to the degradation and elimination of hemicellulose, thereby enhancing cellulose accessibility (Østby et al., 2020). Study by Maffei et al. (2018), where a combination of cellulase and mannanase was used to treat marine microalgae Nannochloropsis sp. The combined use of these enzymes significantly enhanced lipid recovery from the microalgae, increasing the extraction yield from 40.8% to over 73%. This increase was attributed to the removal of amorphous cellulose by cellulase and hemicellulose by mannanase, which improved the accessibility of the cellulose microfibrils.

4.3.2 Genetic Engineering

Recent developments in genetic engineering have led to the creation of microorganisms capable of producing high-yield cellulases with enhanced activity (Ko et al., 2018). In a study by Kaur et al. (2020), a natural variant of Aspergillus niger P-19 was employed to produce a consortium of cellulase and hemicellulase enzymes using solid-state fermentation of rice straw. Another noteworthy example is presented by Ko et al. (2018), where a genetically engineered strain of S. cerevisiae (SXA-R2P-E) was used alongside lignin-modified sugarcane biomass to enhance ethanol production. Recombinant DNA technology enables the production of thermostable and highly active enzymes, improving the overall effectiveness of the process. Genetic modifications also allow for the fine-tuning of enzyme properties, such as pH stability and substrate specificity. As a result, enzymatic cellulose isolation more efficient across a range of biomass types.

4.3.3 Immobilized Enzymes

Enzyme immobilization is another significant advancement that enhances the sustainability and cost-effectiveness of enzymatic cellulose isolation. A study by Sulman et al. (2022) demonstrated the effectiveness of immobilizing cellulase on silica nanoparticles for lignocellulosic biomass hydrolysis. The immobilized enzyme retained approximately 85% of its initial activity after 10 cycles of reuse, indicating high operational stability. Additionally, the immobilized cellulase maintained 90% activity at 60°C for 72 hours, significantly outperforming the free enzyme, which lost 50% of its activity within the same period. Moreover, the immobilized enzyme achieved a 72% hydrolysis efficiency of pretreated corn stover after 48 hours, compared to 55% for the free enzyme, showcasing its enhanced performance in cellulose conversion.

5. RECENT DEVELOPMENT ON CHARACTERIZATION TECHNIQUES

5.1 Structural Analysis

Structural analysis of cellulose is essential for understanding its molecular arrangement, crystallinity, and chemical composition. X-ray diffraction (XRD) is a widely used technique to assess the crystallinity index and distinguish between different cellulose allomorphs, such as cellulose I and cellulose II, by analyzing the diffraction patterns of X-rays interacting with the cellulose structure (Owolabi et al., 2017).

In a study by Samsalee et al. (2023), nanocrsrystalline cellulose extracted using the acid hydrolysis and high presure homogenization (AH+HPH) method exhibited a higher crystal index (CrI) compared to only high-pressure homogenization (HPH). The calculated crystallinity index (CrI) ranged from 62% to 72%, with the AH+HPH12 sample exhibiting the highest value of 72%. Cellulose with the best crystallinity is characterized by a Crystallinity Index (CrI) above 70%. Such a high crystalline structure results in a strong and stable material, making it highly suitable for applications in biocomposites, pharmaceuticals, and other functional materials.

Fourier-transform infrared spectroscopy (FTIR) provides detailed information on functional groups present in cellulose by measuring the absorption of infrared light, allowing researchers to identify hydroxyl, carbonyl, and ether bonds, as well as detect chemical modifications (Kassim et al., 2019). An ideal FTIR spectrum of cellulose typically exhibits characteristic absorption bands that indicate the presence of specific molecular vibrations, such as hydroxyl (O–H), carbonyl (C=O), and ether (C–O–C) bonds.

The O–H stretching band, located around 3300 cm^{-1}, appears sharp and intense when cellulose purity is high, as the removal of lignin during processing narrows the band. The C–H stretching band at 2905 cm^{-1} weakens or disappears after treatments like alkalization, indicating a reduction in hemicellulose content. Additionally, the carbonyl stretching band at 1740 cm^{-1}, commonly associated with hemicellulose, diminishes significantly after alkaline hydrolysis, confirming the degradation of non-cellulosic components.

A notable feature of high-crystallinity cellulose is the increased intensity of the 1440 cm^{-1} band, known as the "crystallinity band," which corresponds to symmetric CH_2 bending vibrations. Furthermore, the absorption bands at 1060 cm^{-1} and 897 cm^{-1} indicate C–O–C stretching related to β-1,4-glycosidic linkages, essential for maintaining the cellulose structure. An increase in the 1160 cm^{-1} band intensity, associated with asymmetrical C–O–C stretching, indicates a higher cellulose content after the treatment process (Pratama, Mahardika, et al., 2024). FTIR analysis, therefore, plays a vital role in confirming the reduction of lignin and hemicellulose while highlighting the enhanced crystallinity of cellulose after chemical treatments. Complementary to FTIR, Raman spectroscopy utilizes laser light scattering to analyze molecular vibrations, offering insights into hydrogen bonding, structural orientation, and crystallinity. Together, these techniques provide a comprehensive understanding of cellulose structure, which is critical for optimizing its applications in biomaterials, textiles, and biodegradable composites (Kouadri & Satha, 2018).

5.2 Morphological Studies

Morphological studies of cellulose are crucial for analyzing its fiber structure, surface characteristics, and nanoscale features. Scanning electron microscopy (SEM) is widely used to examine the surface morphology of cellulose fibers, providing high-resolution images that reveal fiber size, shape, porosity, and structural changes caused by chemical or mechanical treatments. SEM helps assess the efficiency of cellulose isolation processes by identifying impurities and fiber aggregation (Julie Chandra et al., 2016). In contrast, transmission electron microscopy (TEM) offers a more detailed examination at the nanoscale, allowing visualization of cellulose nanofibers and nanocrystals with high clarity. TEM is particularly useful for analyzing the crystalline and amorphous regions of cellulose, as well as fiber alignment and dispersion in composite materials (Gabriel et al., 2021). Together, SEM and TEM provide valuable insights into the structural integrity and modifications of cellulose fibers, which are essential for optimizing their applications in biocomposites, nanomaterials, and functional biomaterials.

5.3 Thermal Properties

Thermal properties play a crucial role in determining the stability and performance of cellulose in various applications. Thermogravimetric analysis (TGA) is a widely used technique to assess the thermal stability and decomposition behavior of cellulose by measuring weight loss as a function of temperature. This method helps identify different degradation stages, including moisture loss, hemicellulose decomposition, cellulose degradation, and char formation. TGA is particularly useful for evaluating the effect of chemical modifications and treatments on cellulose's thermal resistance. High-purity cellulose shows onset degradation around 300°C and maximum decomposition near 350°C, with residual mass less than 30%. Chemical treatments that enhance cellulose purity, such as alkali hydrolysis and bleaching, generally increase the onset temperature and thermal stability, making the material more suitable for applications requiring high thermal resistance (Bacha et al., 2022).

Complementary to TGA, differential scanning calorimetry (DSC) analyzes heat flow changes in cellulose samples to detect thermal transitions such as glass transition temperature (Tg), melting, and crystallization behavior. DSC provides insights into cellulose's phase stability and structural changes under varying thermal conditions. High-quality, crystalline cellulose typically exhibits a Tg around 230°C, Tm around 350°C, and Td above 350°C. Chemical treatments aimed at increasing crystallinity and purity generally result in higher Tg and Tm values, indicating enhanced thermal resistance and stability. These characteristics are essential for evaluating cellulose for biocomposites, packaging, and thermal insulation materials (Al Ragib et al., 2024).

Together, TGA and DSC offer a comprehensive understanding of cellulose's thermal behavior, supporting the advancement of heat-resistant biomaterials for packaging, textiles, and composite applications.

6. DISCUSSION AND FUTURE DIRECTIONS

The growing emphasis on sustainable and eco-friendly approaches in cellulose characterization reflects a shift toward greener extraction and processing techniques. While chemical methods like acid hydrolysis and alkali treatment remain dominant due to their effectiveness in isolating cellulose (Birhanu et al., 2024), enzymatic and biological methods are increasingly recognized for their environmental benefits (Yang & Chen, 2023). These alternative approaches offer advantages such as reduced chemical waste, lower energy consumption, and gentler processing conditions, making them more appealing for large-scale sustainable applications (Sulman et al., 2022). However, limitations such as slow reaction rates, high enzyme costs, and lower extraction yields continue to pose challenges (Kaur et al., 2020).

To address these issues, future research should prioritize the development of integrated techniques that combine chemical, mechanical, and biological methods to improve cellulose purity while reducing environmental impact. Enhancing the scalability of green extraction processes is essential for industrial applications, necessitating advancements in bioreactor technology, enzyme engineering, and process optimization. Additionally, the exploration of cost-effective and efficient methodologies will be critical in expanding the use of cellulose-based materials in industries such as bioplastics, composites, and pharmaceuticals.

Investigating alternative cellulose sources, including agricultural residues and marine biomass, could further promote sustainable production. Economic modeling and life cycle assessments of new cellulose extraction methods will ensure their sustainability and cost-effectiveness, thus supporting the commercial viability of cellulose-based products in diverse industries. By integrating these innovative approaches, cellulose research can drive the development of sustainable and commercially feasible solutions for the future..

Table 1. Advantages and disadvantages isolation cellulose methods chemical, mechanical and enzymatic.

Methods	Advantages	Disadvantages
Chemical	- Highly efficient in removing lignin and hemicellulose - Produces high-purity cellulose - Well establish for industrial applications	- Generates hazardous chemical waste - Requires high energy and extensive water usage - Can degrade cellulose structure, reducing yield
Mechanical	- Effective in reducing cellulose particles size - Maintains cellulose purity without chemical modifications	- High energy consumption - Limited effectiveness in removing lignin and hemicellulose - May require pre-treatment for better efficiency
Enzymatic	- Environmentally friendly, with animal chemical waste - Selectivedegradation of non-cellulosic components - Operates under mild conditions, preserving cellulose integrity	- High enzyme costs limit large-scale application - Slow processing time compared to chemical methods - Enzyme activity in affected by reaction conitions

7. CONCLUSION

This review highlights significant progress in cellulose isolation and characterization, offering insights into evolving research trends through bibliometric analysis. The findings underscore the growing emphasis on developing sustainable and efficient extraction techniques to meet the rising demand for cellulose-based materials. While chemical methods remain widely used for their effectiveness, enzymatic and biological approaches are emerging as environmentally friendly alternatives. However, challenges such as high costs, scalability limitations, and processing efficiency must be addressed. Future research should focus on optimizing hybrid techniques, advancing green methodologies, and exploring novel cellulose sources to enhance sustainability. These advancements will play a vital role in facilitating the board integration of cellulose across multiple applications and supporting the global transition toward eco-friendly technologies.

The isolation and characterization of cellulose from biomass encompass a variety of chemical, mechanical and enzymatic approaches, each offering distinct advantages in terms of yield, purity, and ecological sustainability. Characterization techniques such as Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA) play a vital role in verifying cellulose purity, crystallinity, morphology, and thermal stability. These analytical tools ensure the successful extraction of high-quality cellulose, supporting its applications in bioplastics, composites, pharmaceuticals, and other sustainable materials. As research continues to evolve, integrating advanced extraction techniques with precise characterization methods will be essential in optimizing cellulose production for industrial and environmental applications.

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