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High Purity Lignin from Oil Palm Biomass

Jofry Othman*, Norliza Abd Rahman, Jarinah Mohd Ali & Siti Kartom Kamarudin

Chemical Engineering Programme,

Faculty of Engineering & Built Environment, Universiti Kebangsaan Malaysia, Malaysia

**Corresponding author: jofryothman@petronas.com*

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ABSTRACT

The abundant biomass derived from oil palm exhibits valuable characteristics of lignin and biocellulose, which hold considerable promise for conversion into various products. However, this process necessitates a suitable pretreatment strategy that achieves a balance between effective lignin removal and gentle cellulose extraction, thereby maximizing the potential of these biopolymer components. This research highlights the successful application of lignin and β-cellulose obtained from oil palm biomass in the creation of an innovative biofilm product, emphasizing the efficient use of lignin biocellulose. A promising technique involving bacterial cellulose, which is gaining attention for new applications in the food and medical sectors, is assessed for its effectiveness in biomass pretreatment. The study establishes optimal pretreatment conditions that enhance lignin purity, lignin recovery, and holocellulose yield from oil palm biomass. A lignin purity of 88.2% was achieved, surpassing the 68.9-71.0% purity levels reported in existing literature, while maintaining comparable recovery performance. Furthermore, this method demonstrates a commendable level of cellulosic recovery with minimal disruption to the crystalline structure of cellulose. This results in enhanced lignin purity and recovery, while preserving cellulose crystallinity, which is essential for subsequent product conversion. Nonetheless, it was noted that the current enzymatic method is not fully effective in penetrating the lignin protective barrier during biomass pretreatment. Opportunities for improvement in this approach are identified, particularly in the development of ligninolytic enzymes that can compete with emerging alkaline pretreatment techniques.

Keywords: Oil palm biomass; lignin; biocellulose; biofilm; bacterial cellulose

INTRODUCTION

The high lignin content present in oil palm biomass presents a considerable opportunity for the application of lignin biocellulose in various fields, including its use as antioxidants and for ultraviolet protection (Lee et al. 2020). Many researchers have been investigating techniques to extract compounds from oil palm biomass to achieve a product of high purity; however, they have encountered limited success. The main obstacle is the intricate structure of lignin monomers interlinked with holocellulose in an insoluble network within the biomass polymer, which complicates direct extraction efforts (Shanmugarajah et al. 2019).

As suggested by Mastrolitti et al. (2021), an effective pretreatment method for oil palm biomass should focus on maximizing lignin removal while minimizing any adverse effects on holocellulose. This research has evaluated a viable method for extracting lignin biocellulose and enhancing its properties for diverse applications. An optimal pretreatment condition has been established through a modified alkali-based chemical process, which strikes a balance between efficient lignin extraction and the preservation of cellulose.

This approach results in enhanced lignin purity and recovery, while also maintaining the crystallinity of cellulose, making it suitable for subsequent product conversion. Furthermore, the study highlights the promising potential of utilizing lignin biocellulose derived from biomass in the production of lignin bio-based films and includes a comparative analysis with bacterial cellulose extraction to formulate an effective pretreatment strategy.

COMPARATIVE PERFORMANCE OF BIOMASS PRETREATMENT METHOD

In today's industrial applications, lignocellulosic biomass sourced from agricultural residues, woody crops, forestry residues, and wastepaper produces cellulose and lignin that are commonly utilized in a variety of industrial applications (Potoˇcnik et al. 2023). The current production of lignin relies on the conversion of a restricted supply of plant-based raw materials such as wood pulp and cotton (Chia et al. 2023).

Additionally, as suggested by Rashid et al. (2021) cellulosic d-glucose monomer derived from lignocellulosic biomass is utilized as an ingredient in polymers, composites, membranes, and cellulose fiber products. Although the ease of processing lignin allows for its use in different applications such as resin and adhesive, the limited availability of these raw materials and concerns about sustainability and deforestation have prompted the exploration of lignocellulosic biomass as a second-generation feedstock (Hidayat et al. 2021).

Specifically, oil palm biomass primarily consists of lignin biocellulose, with approximately 51.88% holocellulose (cellulose and hemicelluloses), 31.68% lignin, and 6.69% ash (Nuraishah et al. 2022). A study by Law et al. (2020) suggested the relative quantities of the various biomass cell wall components contribute to it physiochemical properties in crystallinity, tensile strength, modulus, and moisture presence. The cell wall of oil palm biomass is commonly composed of cellulose, which contains polar functional groups, making it hydrophilic. Lignin, however, is mainly located in the interfibrous area, which contributes to the structural integrity of plants (Ajayi et al. 2023).

Accordingly, the different source of lignin provides physical and chemical behavior, and the high recalcitrance of oil palm biomass present difficulty for extracting it closer to the native lignin properties (Chia et al. 2023). Additionally, the presence of silica on the oil palm biomass surface as suggested by Iram et al. (2021) provide a physical barrier to hydrolysis that add to the delignification challenge.

This resulted into the extremely restricted utilization of oil palm biomass for in mere applications such as a fuel for boilers or soil conditioner (Hidayat et al. 2022). Therefore, to discover valuable applications for lignin biocellulose, it is imperative to dissolve the oil palm biomass into separate cellulosic and lignin monomers at high purity levels. This will enable the synthesis of targeted products with high value.

Consequently, this investigation examined the extraction method for obtaining these monomers from oil palm biomass, aiming to achieve a controlled composition and high purity of these molecules. A comparative assessment is conducted to evaluate various delignification methods including physical, chemical pretreatment, physio-chemical, and thermo-chemical techniques to determine their suitability for oil palm biomass pretreatment process.

Among these approaches, alkali-based chemical pretreatment is selected as the preferred method due to several reasons. Table 1 describes the various drawbacks associated with the other pretreatment methods, which include excessive energy consumption, potential for corrosion, production of hazardous substances, variation in product composition, and a slower rate of hydrolysis.

Alkali-based chemical pretreatment method efficiently breaks down the chemical bonds that bind lignin monomer and other compounds within the cell structure (Maha & Teow 2022). It has been discovered by Haqiqi et al. (2021) that this technique enhances the swelling of cellulose, resulting in a higher inner surface area and a decrease in both the degree of polymerization and crystallinity. Hence, this approach holds the capability to attain a beneficial balance between efficient removal of lignin and mild extraction of cellulose, thereby maximizing the utilization of oil palm waste components.

Additionally, the combination of alkaline treatment and ultrasonic sonication has the potential to enhance the extracted yield of lignin, while preserving its structure (Song & Othman 2022). However, further optimization is required to determine the optimal ultrasonic time, thermal stability, and the impact of structural properties on holocellulose.

Nevertheless, if conditions are not applied correctly, the majority of biomass compounds may undergo significant disintegration, resulting in the formation of intricate monomers that pose a challenge for extracting lignin from the broth mixture (Mastrolitti et al. 2021). Additionally, Ajayi et al. (2023) suggests that this process could potentially give rise to unwanted by-products like biochar.

Therefore, to enhance the efficiency of biomass delignification, additional optimization is needed on key parameters such as the type of alkaline chemical used, the quantity of alkalinity present, the duration of pretreatment, and the operating temperature (Mohammad et al. 2020).

TABLE 1. Comparative performance of OPEFB pretreatment method (Ajayi et al. 2023; Mohammad et al. 2020; Song & Othman 2022).

Method	Key Features	Comparative Performance	
		Advantages	Disadvantages
Physical	The OPEFB particle size and crystallinity can be reduced through mechanical grinding. This process involves chipping, grinding, and milling.	The surface area of the biomass can be enlarged, leading to a decrease in the degree of polymerization.	The energy requirement is high, and it is not economically feasible.
Alkali-based chemical	Sodium, calcium, and ammonium hydroxide are typical solvents used in various applications. When exposed to NaOH, cellulose swells, leading to an increase in its inner surface area and a reduction in the degree of polymerization and crystallinity.	Capable of enhancing the digestibility of cellulose and the breakdown of lignin in OPEFB.	The pretreatment conditions are milder with this approach, but it needs to be optimized to minimize prolonged reaction times extended reaction durations.
Ultrasound assisted alkali-based chemical	Comparable conditions to alkali- chemical pretreatment coupled with the use of ultrasonic sonication.	There is a possibility of achieving a greater cleaving effect on the lignin ether bonds. Slight increase with carbon content when compared to alkaline lignin.	A longer duration of ultrasonic irradiation is necessary subject to further optimization. This technique can be applicable for delignification once holocellulose has been removed.
Acid-based chemical	The hemicellulose can be dissolved using this approach, which enhances the accessibility of the cellulose compound.	The use of dilute acids can achieve reasonably high hydrolysis rates, but they produce toxic decomposition products.	This technique is suitable for application with either strong or weak acid but results in the production of inhibitory substances and corrosive environments.
Physico-chemical pretreatment	Pretreatment approach with steam pressurization followed by rapid depressurization can be used. This method can also involve the partial hydrolysis and solubilization of hemicellulose, allowing for the extraction of lignin from biomass.	The use of steam pressure in this process takes a moderate amount of time for processing, and it can redistribute or extract the lignin to a specific extent.	Generates harmful substances and incomplete breakdown of hemicellulose occurs.
Thermo-chemical	The thermochemical procedure involves transforming biomass into a syngas intermediate, , at elevated temperature and pressure. The subsequent chemical conversion of the syngas takes place in a downstream process.	Capable of producing syngas mixture containing controlled ratio of hydrogen and carbon monoxide.	The need for elevated temperatures and pressures in processes such as gasification or pyrolysis leads to high operating costs. Instead of focusing on a specific chemical product, it generates a broader variety of fuel products.
Biological pretreatment	Biological pretreatment utilizes a range of microorganisms to facilitate mild synthetic conditions and eco-friendly environments. Among the microorganisms commonly used are white and soft rot fungi, actinomycetes, and bacteria for breaking down lignin.	The potential of this method lies in its ability to facilitate an extraction process that uses minimal energy, low chemical usage, operates under mild environmental conditions, and involves low investment costs. At the initial phase of proof-of-concept development, there is limited understanding of its capability for sorting or extracting cellulose.	There is not much information about whether this approach is economically viable. Compared to other methods, the main drawbacks are the slower hydrolysis rate and the need for a faster and more efficient delignification process from a new microorganism.

LIGNIN BIOCELLULOSE EXTRACTION AND PROPERTIES

The extraction of lignin biocellulose is conducted out through a modified alkali based chemical process, which is designed to achieve a balance between effectively extracting lignin and balance the effective extraction of lignin with the mild extraction of cellulose. A combined method was used to sequentially extract oil palm biomass lignin and cellulose using optimized parameters, resulting in enhanced lignin purity and recovery, while preserving cellulose crystallinity.

An optimum pretreatment conditions are generated that comprises the use of 4 wt.% sodium hydroxide and the customized operation conditions determined from this study, that maximizes lignin purity, lignin recovery and holocellulose from biomass. A higher lignin purity of 88.2% was produced against 68.9-71.0% purified lignin as reported by (Ajayi et al. 2023; Mohammad et al. 2020). In addition, a high lignin recovery of 83.5-98.7% is achieved from the untreated biomass that demonstrated this method suitability for delignification. This is in comparison to the lower lignin recovery of 65.0-68.9% by using other chemical pretreatment conditions (Ajayi et al. 2023; Parit et al. 2018).

It is also shown that this method provides reasonably high holocellulose recovery of 59.0-63.2% whilst minimize alteration to the crystalline cellulose structure. The high content of extracted cellulose may also be a result of the dissolution of additional components in the sodium hydroxide solution. In comparison, the use of solvent for dissolving cellulosic into sugar monomers such as N-methylmorpholine N-oxide (NMMO) increase the pretreatment cost, lowering the recovery yield and limiting the direct application of cellulose (Haqiqi et al. 2021).

Specifically, the alkali based chemical method performs the cleavage of ester bonds that connect lignin and hemicellulose, resulting in an augmentation of biomass surface area, as proposed by our previous research (Othman et al. 2024). This study also found that this process causes most of the soluble lignin to migrate into a weak sodium hydroxide solution, leaving cellulose and hemicellulose with crystalline structure intact as solid byproducts.

Dissolving lignin or lignin salt in an alkaline solution leads to greater lignin removal compared to dissolving it in acid pretreatment (Pasma et al. 2019). As a result, it is demonstrated in this study an optimized process that provide balance between the lignin recovery rate, achievable lignin purity and carbon content. Accordingly, this study presents an optimized process that demonstrates a balance between the recovery rate of lignin, the purity of lignin, and the carbon content.

The holocellulose extracted from biomass was found to exhibit similarities with complex cellulosic carbohydrates, featuring polymer chains linking unbranched β-1,4-polyacetal cellobiose, as proposed by Girard et al. (2024). A combined pretreatment system was developed to acidify the biomass fiber with the use of a sodium chloride solution. By optimizing the fiber to solvent ratio parametrically using a chemically stable solvent under controlled conditions, non-degradable β-cellulose was effectively generated.

The effective use of lignin biocellulose was demonstrated by utilizing biomass lignin and β-cellulose to produce biofilm embedded with lignin in our earlier study (Othman et al. 2024). Nevertheless, there have been no reports in the literature about the conversion of raw oil palm biomass into biofilm or the alteration of biomass derived cellulose. One notable exception is the research conducted by Ajayi et al. (2023) where efforts were made to extract, analyze, and modify biomass cellulose in a hydrophobic manner.

As suggested by Girard et al. (2024), the key biofilm product properties on water solubility and chemical reactivity are primarily dependent on the degree of substitution (DS), and to lesser degree are the molecular weight and carboxymethyl substitution position in the polymer chain.

The previous study by Song & Othman (2022) has thoroughly examined the properties of lignin biofilm characterization, demonstrating its similar performance to commercially available biofilm. This has created possibilities for converting lignin biocellulose into a novel and innovative product for a range of practical uses.

MATERIAL AND METHODS

QUALITY IMPROVEMENT

The varying compositional properties of oil palm biomass necessitate the implementation of a control system to maintain consistency in the physiochemical properties of the extracted compound. The raw material is sourced from a palm mill in Klang Valley, where it undergoes a 24-hour crushing and sieving process to eliminate moisture content. A control mechanism is then enforced to ensure uniformity in the received biomass batches, focusing on aspects like hydrothermal pretreatment for moisture reduction, shredded fiber size, coarse impurities, and density.

The fresh biomass, with approximately 70% moisture, was subjected to a drying process at 105°C to decrease its moisture content by 8–10% by weight. Further measures were implemented to store the fresh biomass at temperatures ranging from 36-45°C to prevent decomposition and mold formation. Subsequently, the characterization of the fresh biomass was carried out to evaluate its physical properties such as ash, moisture, and metal impurities.

This analysis was performed in compliance with the ASTM E1755-01 (2020) standards for assessing ash content in biomass, which also included an evaluation of the inorganic and mineral constituents of the biomass. The organic composition and metal content were analyzed using a carbon, hydrogen and nitrogen (CHNS) elemental analyzer (Thermo Scientific, FlashSmart) and inductively coupled plasma optical emission spectrometry (Perkin Elmer Optima, DV5300). Additionally, the microstructural characteristics of biomass were examined utilizing a field emission scanning electron microscope (Hitachi U8000, UHR FE-SEM cryoemission).

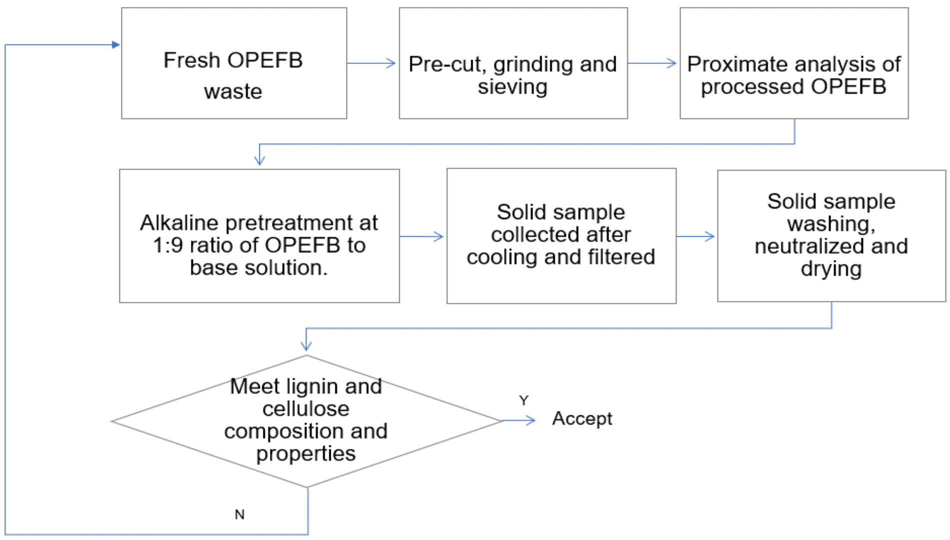

FIGURE 1. Quality process flow

EXTRACTION METHOD

The biomass that had been pretreated hydrothermally was subsequently subjected to an alkaline pretreatment using a diluted solution of sodium hydroxide, as shown in Figure 2 following the guidelines outlined in reference (Haqiqi et al. 2021). The resulting biomass residue was filtered, neutralized, and dried at a moderate temperature until a constant mass was achieved. This process yielded crude cellulose, which was further characterized through bleaching to holocellulose, comprising α-cellulose and hemicellulose.

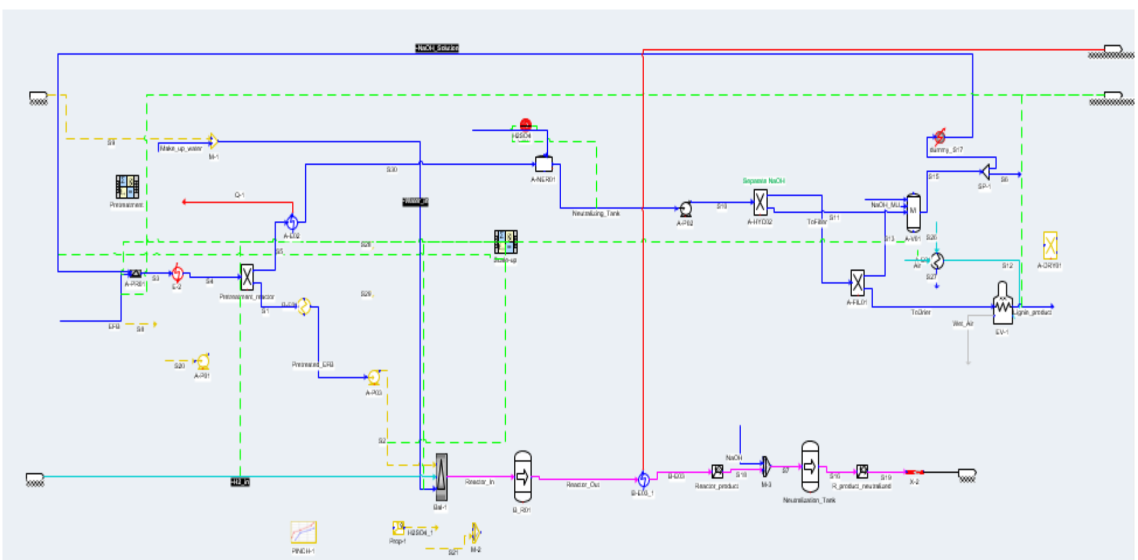

FIGURE 2. Lignin extraction process flow

The characterization tests conducted considered the robust polymer structure of cellulose, known for its chemical stability and insolubility in both polar and nonpolar solvents. Subsequently, the synthesis of biofilm from lignin biocellulose involved the esterification of α-cellulose hydroxyl groups with chloroacetic acid in the presence of an alkaline solution. The experimental trials were conducted to experimentally verify the optimum biomass pretreatment conditions, followed by further testing to ensure the reproducibility of the results. As a result, a procedural model was developed to improve the permeability and wetting characteristics of biomass, optimizing the pretreatment process to reduce lignin levels without compromising the cellulose properties.

The pre-treated biomass underwent neutralization with diluted sulfuric acid to separate lignin from the solution for further refinement. Biomass lignin exhibits similar behavior to lignocellulosic biomass as suggested by Chia et al. (2023), particularly in terms of how its dissociation depends on pH levels. The purified lignin is then subjected to centrifugation and lyophilization before undergoing characterization tests. The remaining solid residue of biomass was filtered, neutralized, and dried at a moderate temperature to create a dry raw cellulose mass. This mass was then bleached to produce cellulosic monomers, with alpha cellulose subsequently extracted for physiochemical analysis.

EXTRACTION METHOD

The lignocellulose extraction and purification parameters were determined with Design Expert v12 to generate the optimal acid concentration for maximizing compound recovery. This found a balance between compound recovery rate, attainable purity, and carbon content in the pretreatment process to improve monomer solubility in the solvent solution. The purity of the extracted monomer was assessed using Ultraviolet–visible spectroscopy (Hach DR6000 spectrophotometer) in accordance with the method previously described by (Haqiqi et al. 2021).

The absorptivity was determined using the Beer-Lambert law [3]:

$$\varepsilon = \frac{A}{(c \times l)} \qquad (1)$$

The absorptivity ε is represented in L $[g.cm]^{-1}$, while A stands for the absorbance (a.u.), c represents the concentration of the sample $(g.L)^{-1}$, and l is the pathlength of the measured cell (cm). Following this, the molecular weight of the monomer is established through gel permeation chromatography (Viscotek Gpcmax, Ve2001). The physical properties, including the mean molecular weight (Mw), mean molecular weight Z (Mz), mean molecular weight number (Mn), and polydispersity, are then obtained from the resulting chromatogram.

The degree of substitution of biofilm is subsequently determined based on the previous studies by (Hidayat et al. 2022):

$$DS = \frac{162\, x \frac{0.3\, x\, V}{m}}{1-80\, x \frac{0.3\, x\, V}{m}} \qquad (1)$$

The molecular weight of the anhydrous glucose unit is 162, with a net increment of 80 in the anhydrous glucose unit for every substituted carboxymethyl group. V represents the acid volume, while m stands for the mass of biofilm. Further properties were analyzed, including the thermal durability of existing film and biomass film using thermogravimetric analysis (Mettler Toledo Thermal Analysis). The analysis was carried out in an inert nitrogen environment to avoid sample combustion, ranging from room temperature to 900°C with a heating rate of $10\frac{°C}{min}$.

According to Hidayat et al. (2022), bacterial cellulose exhibits a structure similar to that of lignin biocellulose; however, they differ significantly in their physical and chemical properties. This study suggests that the restricted investigation into the identification of bacteria that can degrade biomass cellulose is primarily attributed to the elevated lignin content. It was observed that the concentration of lignin hinders the enzymes necessary for cellulose hydrolysis during the extraction process. Nevertheless, acid hydrolysis pretreatment is frequently favored as a method to remove lignin and enhance the surface area of holocellulose, thereby facilitating more efficient enzymatic reactions.

RESULT AND DISCUSSION

QUALITY IMPROVEMENT

It was determined that the biofilm achieved uniform moisture content at this stage and was subsequently sampled for compositional analysis, as detailed in Table 2. The moisture content of biofilm raw materials can be controlled to less than 8-10% by weight, with coarse dirt kept to a minimum of less than 3% by weight, and fiber size ranging from 1-3cm, enabling precise control of pre-treatment parameters during processing, and enhancing optimization.

By loading biofilm under more stable conditions during pretreatment, the impact of mold decomposition on pretreatment performance can be avoided. Additionally, the reduction of dry fiber size allows for a higher fiber content, leading to improved biomass loading efficiency and a smaller pretreatment reactor.

TABLE 2. Biomass quality control parameters

Variables	Controlled Specifications	Remarks
Moisture content	8-10 wt.%	Storage temperature of 36-45 ℃ for 3 days can inhibit degradation and mold formation.
Coarse dirt (impurities and ash)	Maximum of 3 wt.%	Able to be removed by water washing
Press shredder	1- 3 cm	Press shredder
Density	0.7- 1.55 $\frac{g}{cm^3}$	Controlled density range allow for improved pretreatment control

SEM images of oil palm waste biomass samples at x400 magnification can be observed below. The images reveal the presence of round-shaped spiky silica extrudates on the fiber surface, covering a significant portion of the surface area. Previous study by Katahira et al. (2018) has indicated that these silica deposits serve as crucial physical obstacles for enzymatic hydrolysis, hindering access to the holocellulose surface. Consequently, an excessive delignification may result in harm to the biomass surface, such as destroying the cellulose structure and reduce its size, as demonstrated by previous research (Chia et al. 2023; Katahira et al. 2018).

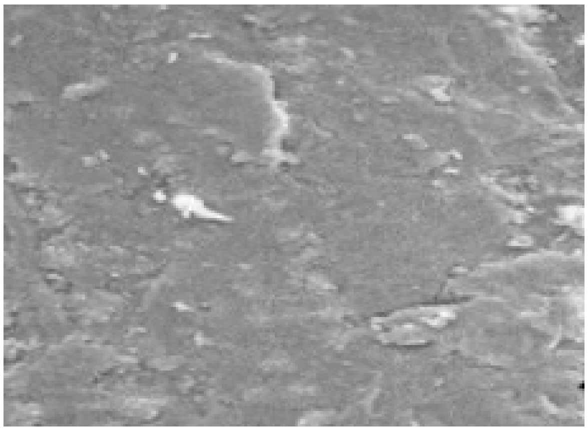

FIGURE 3. SEM image on the fiber structure at x400 magnification

PRODUCT PURIFICATION

This study revealed that the most efficient ligno biocellulose recovery was achieved through a two-step alkali based chemical method and acidic based precipitation. By acidifying the uniform solution of pretreated biomass following the procedure outlined in Haqiqi et al. (2021), complete precipitation of lignin from biomass was achieved. The two-step acid precipitation procedure conducted in this study facilitated the production of exceptionally pure lignin, as the lignin particles were able to increase in size at pH 6 before undergoing additional precipitation at a lower pH, where impurity levels were at their peak.

The extracted lignin purity from Ultraviolet–visible spectroscopy was determined by the absorbance spectrum measured at 280 nm, falling within the 0.3 – 0.8 a.u range (with a dilution factor of around 1:2500). Consequently, a workflow model was established to enhance the accessibility and wettability of biomass, optimizing the pretreatment process to decrease lignin content without compromising cellulose properties.

The analysis of the molecular weight through gel permeation chromatography on the isolated lignin specimen revealed Mn, Mw, and Mz values of 2,558 Da, 2,908 Da, and 3,483 Da, correspondingly, along with a suitable polydispersity index of 1, as detailed in the earlier study (Song & Othman 2022). Consequently, the alkali-treated cellulose, following the described method, resulted in the production of α-cellulose, which was identified as a viable source material for the creation of biofilm.

Furthermore, the thermogravimetric analysis of this film demonstrated favorable thermal stability when subjected to incremental temperature increases from ambient temperature to 900°C. In general, uniform patterns in thermal properties were noted for all films in line with the results by (Girard et al. 2024).

EXTRACTION METHOD OF BIOMASS CELLULOSE

This study found that a viable extraction method for cellulose from biomass has not been established. This is primarily attributed to the significant obstacles faced during pretreatment, particularly in addressing the inherent resistance of oil palm waste biomass. One major challenge involves eliminating silica bodies present on the surface of biomass, which hinder the hydrolysis process.

One potential method involves utilizing T. reesei bacterial strains that have shown moderate effectiveness in extracting biomass cellulose and lignin, as suggested by

(Swingler et al. 2021). Most microorganisms are observed to achieve maximum enzyme production between 24 to 72 hours. Nevertheless, this process exhibits a slower reaction rate when compared to alkali-based chemical methods and presents challenges in enzyme removal (Perera et al. 2023).

The use of lignin degradation enzymes in most of the research is restricted to the degradation of post-extracted biomass lignin into potential derivatives. Parit et al. (2018) conducted a study on the organosolv approach for extracting biomass lignin and cellulose, resulting in 15.1 ±1.3 wt.% and 63.3 ±1.8 wt.% yields, respectively. A similar method was employed by Mohammad et al. (2020) to enzymatically hydrolyze biomass lignin into glucan-rich, lignin-rich, and hemicellulose.

Lignin degradation enzymes face significant obstacles, such as the efficient extraction of lignin in biomass polymer network by microbial action and the elimination of silica residuals on the fiber surface. Moreover, the high macromolecular nature of lignin necessitates its separation into low molecular weight monomers before it can be utilized in specific products, requiring high-purity fractions for high-value applications. Similarly, alpha cellulose also requires attention to microbial activity, albeit to a lesser extent.

Hence, potential areas for future research in enhancing the production of ligninolytic enzymes lie in optimizing fermentation media, precise pH regulation, and synthesis parameters. There is potential for pretreatment strategies that merge chemical techniques with bacterial cellulose and compatible enzymes capable of tolerating high temperatures ranging from 45-80°C, as well as a broad pH spectrum.

CONCLUSION

The reduction in size of biomass dry fiber has been demonstrated to increase fiber content, resulting in enhanced biomass loading efficiency and a smaller pretreatment reactor. This streamlines the purification process, yielding 62.1% alpha cellulose and 88.2% lignin by weight. The refined extraction technique minimizes impacts on cellulose properties while maximizing lignin output.

As a result, a cellulose structure akin to that of native biomass cellulose can be attained. The holocellulose obtained from biomass displays resemblances to intricate cellulosic carbohydrates. When combined with the extracted lignin, a proven application in biofilm has exhibited promising feasibility.

This study found that an effective extraction method for bacterial cellulose from oil palm waste biomass has not been developed. This is mainly due to the major difficulties encountered during the pretreatment process. The latest progress in this area shows a slower rate of reaction in comparison to chemical methods based on alkali, and it poses difficulties in removing enzymes.

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DECLARATION OF COMPETING INTEREST

None.

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