



Greener pretreatment and the characterization of neem and babul tree barks for lignin extraction

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ABSTRACT

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Lignocellulosic biomass is one of the most commonly produced agricultural wastes, with a rich composition of cellulose, hemicellulose, and lignin. Despite being one of the world's primary sources of energy, it is not adequately utilized. Therefore, the current research focuses on using a locally available biomass resource to extract lignin from plants through an acid pretreatment method. Two distinct biomasses, consisting of babul and neem tree barks (NTB), were used to extract lignin. Following pretreatment, proximate and ultimate analyses, Energy Dispersive X-ray Spectroscopy (EDS), Scanning Electron Microscopy (SEM), and Thermogravimetric Analysis (TGA) were used to characterize the biomass and the extracted lignin. Compared to other biomasses, babul tree bark (BTB) showed the highest concentration of lignin recovery, whereas NTB had the lowest sulfur content. After 5 hours of NTB laboratory experimentation, a 14% yield of lignin, the minimum lignin yield compared to BTB (15%), was obtained. When compared to NTB biomass, it is evident that BTB produces more lignin. TGA results show that 10% of the original mass of the NTB lignin was lost due to decomposition, evaporation, or other thermal transformation processes. The extracted lignin, which has a higher phenolic content than commercial lignin, can be utilized in future applications.

Contribution/Originality: The utilization of Babul and neem tree bark for lignin extraction has not been explored in previous studies. Both biomasses contain a significant amount of lignin. This study highlights an additional source for lignin recovery. The methodology considers the specific characteristics of the feedstock.

1. INTRODUCTION

Lignocellulosic biomass, derived from various plant sources, is now an auspicious feedstock for sustainable biofuel and bioproducts production due to its abundance and renewability. Neem (*Azadirachta indica*) and Babul (*Acacia nilotica*) trees are two such sources of lignocellulosic biomass, possessing significant potential for lignin extraction (Akhter et al., 2023), which is a key component in the development of bio-based materials and biofuels. The efficient extraction of lignin from these tree barks is a crucial step in harnessing their full potential for bioenergy applications. The sustainable utilization of lignocellulosic biomass necessitates the development of environmentally

friendly and efficient pretreatment methods (Akram et al., 2023; Yang et al., 2023). Lignin is an essential structural polymer in plant cell walls that provides rigidity, strength, and resistance against microbial and environmental degradation. It protects cellulose and hemicellulose from enzymatic attack, making it vital for plant survival and growth. Industrially, lignin is a major renewable source of aromatic compounds, which can be converted into bio-based chemicals, fuels, adhesives, and polymeric materials. Its antioxidant and UV-absorbing properties also make lignin valuable for applications in coatings, packaging, and pharmaceuticals. As biorefineries advance, efficient lignin valorization is becoming the fulcrum of improving overall economic and environmental sustainability in biomass utilization. The term "greener pretreatment" encompasses methods that reduce environmental impact, minimize chemical usage, and improve overall process sustainability. Several studies have examined various pretreatment techniques (Siddique, Mengal, Khan, Khan, & Kakar, 2023), including chemical, physical, and biological methods, to facilitate lignin separation from cellulosic and hemicellulosic fractions. The majority of such methods often involve harsh chemicals, high temperatures, and energy-intensive processes, which can be environmentally detrimental and economically unsustainable.

Recent advancements have underscored the importance of adopting greener pretreatment strategies, despite existing challenges. Existing approaches are known to enhance lignin extraction efficiency and minimize the environmental footprint associated with the process (Watkins, Nuruddin, Hosur, Tcherbi-Narteh, & Jeelani, 2015). Researchers are striving to align lignin extraction processes with the principles of green chemistry and the circular economy for the benefit of sustainability. In the context of neem and babul tree barks (BTB), the literature reveals a growing interest in their potential as lignocellulosic feedstocks. Previous studies have explored the chemical composition of such barks, including cellulose, hemicellulose, and lignin content. Other researchers have additionally investigated various pretreatment methods, such as steam explosion, acid hydrolysis, and enzymatic treatments, to extract lignin from the aforementioned sources (Ab Rahim et al., 2018; Yimtrakarn, Kaveevivitchai, Lee, & Lerkkasemsan, 2022). Clearly, former studies have yielded valuable insights, but there remains a need to further investigate and optimize greener pretreatment techniques tailored specifically to neem and BTB. The present study contributes to the existing literature by applying a greener pretreatment approach for extracting lignin from neem and BTB. It adopts environmentally friendly processing conditions and characterizes the extracted lignin using EDS, SEM, and TGA, as in several studies, including (Verma, Kumar, Kaushik, Sirohi, & Lai, 2025). The research's primary contribution is to demonstrate sustainable lignin recovery from these underutilized biomasses. Thus, we contributed to the existing body of knowledge by evaluating and refining greener pretreatment methods for the two biomass species and characterizing the extracted lignin. The bioenergy sector will benefit significantly from this study's findings.

2. LITERATURE REVIEW

The challenges associated with lignin pretreatment, extraction, and depolymerization must be addressed in order to fully realize its value in biomass utilization (Garedew et al., 2020). Greener pretreatment of biomass involves breaking down lignocellulosic structures using environmentally friendly and energy-efficient methods. It aims to reduce or eliminate harsh chemicals, lower reaction temperatures, and minimize waste generation. In view of that, Den, Sharma, Lee, Nadadur, and Varma (2018) review milder, greener oxidative pretreatment strategies to improve selective depolymerization and support sustainable biorefinery development. Enzymatic hydrolysis, steam explosion, ultrasonication, organosolv, alkali treatment, and microwave-assisted processing are commonly used to achieve effective biomass deconstruction with reduced environmental impact (Ardhiansyah et al., 2024). They help to preserve the natural composition of cellulose, hemicellulose, and lignin for high-value downstream applications. Enzymatic hydrolysis is advantageous because it converts pretreated lignocellulosic biomass into fermentable sugars under mild, environmentally friendly conditions without generating toxic byproducts (Capolupo & Faraco, 2016). Steam-explosion pretreatment uses high-pressure steam followed by rapid depressurization to break apart the dense

lignocellulosic structure (Negro, Álvarez, Doménech, Iglesias, & Ballesteros, 2020). It increases enzyme accessibility and enables high fermentable sugar recovery for downstream biofuel production. Microwave-assisted hydrothermal pretreatment enables acid-free, energy-efficient recovery of fermentable sugars from brewer's spent grain (López-Linares, García-Cubero, Lucas, González-Benito, & Coca, 2019). However, Verma et al. (2025) compare microwave and ultrasound green pretreatment methods for extracting hemicellulose (xylose) from cauliflower waste, where microwave pretreatment achieves a higher yield and lower energy consumption. Greener pretreatment basically supports the principles of green chemistry and sustainable resource utilization. According to Norrrahim et al. (2021) lignocellulosic biomass is a cheap and abundant resource; its utilization is limited by the need for pretreatment. The same author reviewed greener physical and biological pretreatment methods as alternatives to conventional chemical processes and discussed their effects on bioproduct conversion. Biological pretreatment uses microorganisms or enzymes to selectively degrade lignin and weaken the lignocellulosic structure under mild, chemical-free conditions (Anu, Kumar, Singh, & Singh, 2023). Green treatment methods also enhance the feasibility of converting biomass into renewable fuels, chemicals, and bioproducts. In Dharmaraja et al. (2023) and Roy, Rahman, and Raynie (2020), the novelty lies in reviewing and emphasizing new green pretreatment techniques, including ionic liquids (IL), deep eutectic solvent (DES), supercritical carbon dioxide explosion (Sc-CO₂), and co-solvent enhanced lignocellulosic fractionation (CELFF) processes, that improve lignocellulose digestibility. ILs act as green solvents that disrupt the lignocellulosic structure and dissolve biomass components without the harmful waste, volatility, or toxicity associated with conventional acid or alkaline pretreatments (Gunny & Arbain, 2013). DES acts as a low-cost, green solvents that disrupt the crystalline structure and increase the surface area of lignocellulosic biomass, thereby improving its accessibility for hydrolysis and conversion into fermentable sugars (Gunny, Arbain, & Jamal, 2017). Demonstratively, Sun et al. (2023) present a green pretreatment strategy combining hydrothermal and alkaline DES to efficiently fractionate bamboo and achieve high yields of fermentable sugars and value-added hemicellulose products. DES combined with solid-liquid extraction provides a green, efficient, and selective method for recovering valuable compounds from biomass (Wardani et al., 2024). Also, Morán-Aguilar, Costa-Trigo, Aguilar-Uscanga, Paz, and Domínguez (2024) discovered that acid-based deep eutectic solvents (ADES), e.g., the choline chloride-acetic acid system, effectively pretreat sugarcane bagasse under mild conditions to enhance polysaccharide digestibility. Supercritical fluids, particularly supercritical CO₂ and water, act as green, tunable media that can effectively disrupt lignocellulosic structure and enhance hydrolysis (Kumar, Kermanshahi-pour, Brar, & Brooks, 2021). Many solvents that could solve the prevailing issues have been reported. Green solvents under microwave irradiation efficiently absorb and convert microwave energy into heat, enabling rapid lignocellulose breakdown and enhanced separation of biomass components (Wang et al., 2024). Natural hydro-low-transition-temperature mixtures (NH-LTTMs) solvent form strong hydrogen-bonding networks that disrupt the lignocellulosic structure, to boost biomass dissolution and lignin extraction under mild conditions (Yiin et al., 2018). Glycerol, when mixed with glycerol carbonate, helps assist biomass delignification and fiber defibrillation under mild acidic conditions, which trigger high glucan digestibility and glucose yield (Zhang, Rackemann, Doherty, & O'Hara, 2013). Barks are highlighted as promising but complex lignocellulosic feedstocks that require tailored pretreatment strategies due to their unique lignin composition and extractive content (Guan et al., 2025). Barks are more suitable for pretreatment-based biorefinery pathways than direct reductive catalytic fractionation. Tree bark lignin is chemically diverse, with varying proportions of syringyl, guaiacyl, and p-hydroxyphenyl units, and different levels of β -ether and condensed linkages (Neiva et al., 2020). They are a valuable source of phenolic compounds, which influence their suitability for specific valorization strategies. Findings of Karnaouri, Rova, and Christakopoulos (2016) show that pretreatment alters the bark's structure and composition by selectively removing or modifying components, with organosolv enriching suberin while hydrothermal and steam explosion cleave polysaccharides. Hot water extraction followed by organosolv pretreatment alters the chemical composition and antioxidant properties of bark-derived lignin, and according to Grzybek, Sepperer, Petutschnigg, and Schnabel (2021) enables the recovery of high-value lignin

fractions. Since starch supplies for bioethanol become limited, forest biomass (including wood, bark, and residues) requires effective pretreatment for sugar release (Hu, Heitmann, & Rojas, 2008). Hu et al. (2008) also state that steam explosion with acetic acid and ammonia fiber explosion are promising strategies for future commercialization.

3. MATERIAL AND METHOD

3.1. Materials

Different agricultural waste feedstock, e.g., neem tree bark (NTB) and babul tree bark (BTB) biomass, were employed in this study. NTB and BTB were purchased from Jamshoro, Sindh, Tandojam, Pakistan. Chemicals such as sodium hydroxide (NaOH), sulfuric acid (H_2SO_4), beakers, and funnels are available at a scientific supply store near Jamshoro, in Pakistan's Haidar Chowk scientific store. Fresh biomass samples were cleaned and dried for 10 days to reduce their water content to 10% (wet weight basis). To dry the samples, the material was cut into various sizes for the grinding apparatus and processed using a grinding machine (universal UMS Dec mill). The wheel, auto-balance mechanism, and operator handling ensured the production of tiny particle components. Several particle sizes were created by sieving the samples after grinding the biomass. The ideal particle size for grinding feedstock is 250 nm. Before being employed for experiments, the biomass material was dried in an oven for 24 hours at 70°C. For the tests distilled water was used. Nonetheless, all biomass samples were weighed, filtered through filter paper, and stored in bottles until further processing. The designed methodology in Figure 1 has proven to extract lignin from biomass.

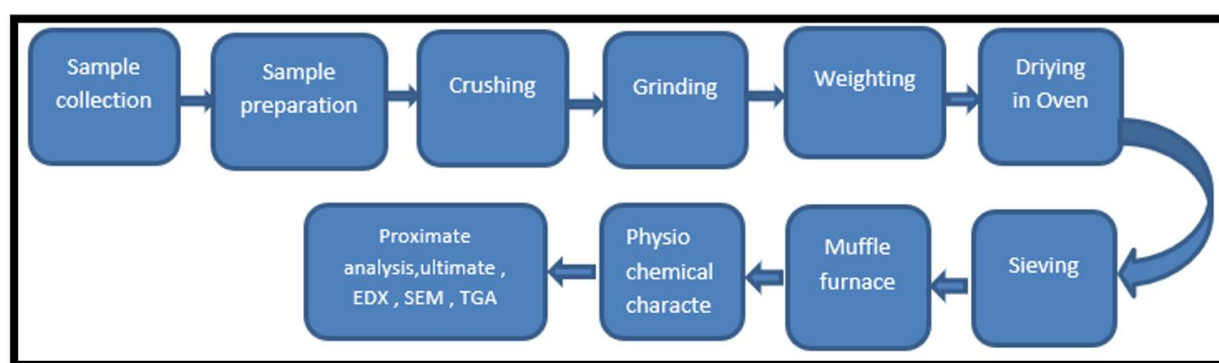


Figure 1. Experimental research methodology setup for the extraction of lignin from the selected biomass.

3.2. Extraction of Lignin

Fifty grams of biomass were taken and added to a beaker containing 1000 mL of distilled water. NaOH was used in this process. After pretreatment, the sample was kept in an oven set at 110°C for 3 hours. The pretreated solution was filtered using filter paper into a beaker. The delignified solid was washed with distilled water. A magnetic hot plate stirrer was used for the solid-liquid ratio (1:20), while a magnetic stirrer was kept in the beaker for agitation. In the 4th hour, the temperature was increased to 120°C. The lignin from the pretreated biomass was recovered using filter paper. Sulfuric acid was added dropwise using a pipette to adjust the pH value to 1 before precipitating the lignin. The lignin residue was separated, and distilled water was used until the neutral pH of 7 was achieved. The extraction process aimed to isolate lignin (Erdocia et al., 2021; Yimtrakarn et al., 2022). Finally, the lignin sample was kept in an oven for drying at a temperature of 105°C. After drying the sample, the extracted lignin was kept in plastic bags for characterization.

3.3. Proximate Analysis

Moisture content, volatile matter content, and ash content of the biomass under study were determined as described in Efetobor, Ikpeseni, and Sada (2022) for elephant grass in conjunction with other standard methods

3.3.1. Moisture Content

Percent moisture content was determined using the oven test standard method (ASTM D2867-09). The sample was placed inside a dry and properly weighted container. It was dried in a prepared oven at a temperature between 130–145°C. After drying, it was removed from the oven and left to cool at room temperature before undergoing a second weigh-in. The percent difference, known as the moisture content of the sample, was thus determined.

3.3.2. Volatile Matter Content

Using the approved test method, ASTM D5832-98 (ASTM, 2021), the proportion of volatile matter in activated carbon was calculated. A covered, weighted crucible containing 1 g of sample was heated in a muffle furnace to 950°C for 7 minutes. Noting that the % difference is what is referred to as volatile matter, it was calculated after cooling the biomass sample at room temperature.

3.3.3. Ash Content

To calculate the ash content, the muffle boiler method test (ASTM D 2866-94) was employed (ASTM International, 2018). The cup (or crucible – China) was weighed with a weight balance before being filled with the activated carbon of a 1 g sample. It was cooled to room temperature in a desiccator after spending some time in the electric furnace at a set temperature of 600°C until it reached the desired weight. The percentages of ash, volatile matter, and moisture content have to be multiplied. The result had to be subtracted from 100 to get the necessary fixed carbon.

3.4. Ultimate Analysis for NTB and BTB

Raw sample weighing 350 mg was subjected to a final analysis using oxygen as the gas and the SC 832 LECO as the model. The biomass sample was sieved to obtain a particle size of 150 microns and dried at 110°C for 1 hour to obtain results on a dry basis. Further comprehensive examination of the raw BTB and NTB samples reveals the amount of elemental carbon (C), hydrogen (H), nitrogen (N), sulfur (S), and oxygen (O) in the two, in accordance with the description of Singh, Mahanta, and Bora (2017). Whenever it comes to basic ingredients, the babul tree has the least amount of S and the most C. Almond shells have a higher O content than BTB. Using acid washing will be able to raise the concentrations of C, H, and O in lignocellulosic material by decreasing the level of N and S.

3.4.1. EDS Lignin Characterization

EDS stands for Energy Dispersive X-ray Spectroscopy. It is an analytical technique used in materials science and various scientific disciplines to determine the elemental composition of a sample. EDS is typically employed in conjunction with scanning electron microscopy (SEM). When an SEM is built appropriately, the electron beam excites the atoms' surfaces, causing them to produce certain X-ray wavelengths that reveal the atomic structure of the elements. An energy-dispersive detector, a solid-state device capable of discriminating between various X-ray energies, analyzes the X-ray emissions. Subsequently, the appropriate elements are identified, revealing the atomic composition of the specimen's surface. The EDS apparatus can be used to detect the presence of lignin. EDS, also referred to as energy-dispersive X-ray spectroscopy, is a method for analyzing a material's surface composition. This method was used to determine the elemental makeup of the lignin sample made from biomass. Elemental analysis was performed on two samples, including the Babul tree bark and neem tree bark, following the method described by Stark, Yelle, and Agarwal (2016).

3.4.2. SEM Analysis of Lignin

SEM is an imaging technique used to study the surface morphology of a wide range of materials, including lignin, which is a complex organic polymer present in the cell walls of biomass. SEM analysis is a valuable tool in lignin

research and was employed in this study.

3.4.3. Thermogravimetric Analysis (TGA) of Lignin

Based on the technique demonstrated by Tao, Li, Li, and Wu (2016) Perkin-Elmer Pyris V-381 thermal gravimetric analyzer was used to measure the thermal decomposition of the sample lignin. The lignin sample was placed in an aluminum pan. It can be subjected to heat within a temperature range of 40-550°C under a nitrogen blanket. The better and more accurate temperature was found. It was then used for the thermal stability of lignin found in the biomass.

4. RESULT AND DISCUSSION

4.1. Analysis of Feedstock for the Lignin Extraction

Proximate analysis as well as ultimate analyses of the feedstock were carried out in the laboratory. The outcomes are presented in Tables 1 and 2. These findings demonstrate that biomass can be produced from materials including BTB and NTB. Proximate analysis is the process of determining the amounts of ash, volatile matter, fixed carbon, and moisture content using specialized techniques. It is feasible to determine what percentage of biomass burns as inorganic waste (ash), volatile matter, and solid-state (fixed carbon) by carefully inspecting the biomass. Babul and neem tree barks are all accepted as biomass in this lab. Table 2 displays the outcomes of the final analysis of the methods specified for the determination of C, H, N, S, and O percent. BTB has higher C content compared to NTB. The heating value is increased by the C content. Sulfur increases the amount of SO_x in the atmosphere, while N will causes NO_x concentration. Here, the two samples of biomass, which are BTB and NTB, were subjected to laboratory testing. The alkali method was used to treat biomass. Based on these findings, Table 1 demonstrates that it was possible to extract lignin to determine the relative amounts of moisture, volatile matter, ash, and fixed carbon in each biomass. Table 3 demonstrates that a 15% BTB yield for 5 hours at 120°C is an excellent type of yield. On the other hand, NTB shows a minimum content yield of 14%.

Table 1. Samples of proximate analysis.

Biomass	Moisture content (%)	% Volatile matter	% Ash	% Fixed carbon
NTB	3.77	76.08	1.76	17.14
BTB	4.23	75.69	2.98	18.07

Table 2. Ultimate analysis of processed biomass raw samples (Pretreated on dry ground).

Biomass	C	H	N	S	O
NTB	52.79	4.7	0.65	0.17	41
BTB	55.71	4.3	0.18	0.14	39

Table 3. Yield of lignin from NTB and BTB at 120°C cover various periods.

Biomass sample	Temperature (°C)	Time (Hour)	Liquid solvent Conc.	Raw material to liquor ratio	Speed of stirrer	Lignin yield (wt%)
BTB	120	5	2%	1:20	Constant	15
		4				11
		3				9
NTB		5				14
		4				10
		3				8

The graphs in Figure 2 respectively depict the time and lignin yield as well as the extraction of lignin from various biomass-to-liquid ratios. BTB has 6.44% sulfur, whereas NTB contains only 2.73% sulfur, which is less than

BTB. The lignin yield analysis is performed for each experimental condition, and the findings are displayed in Figure 2.

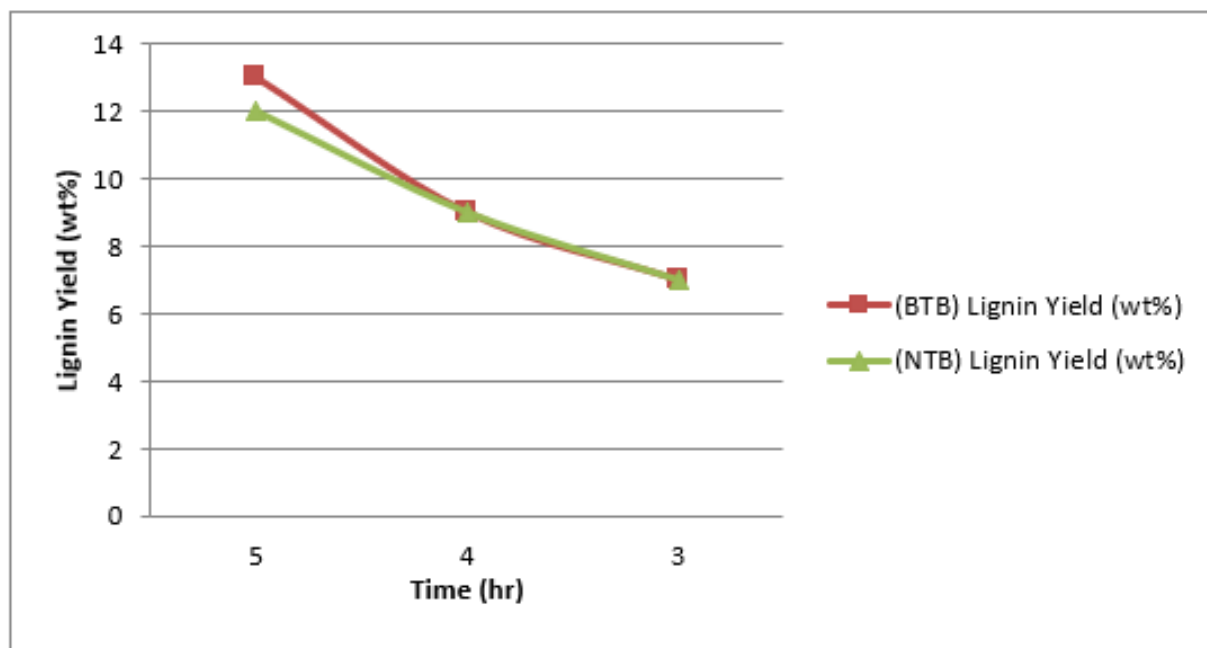
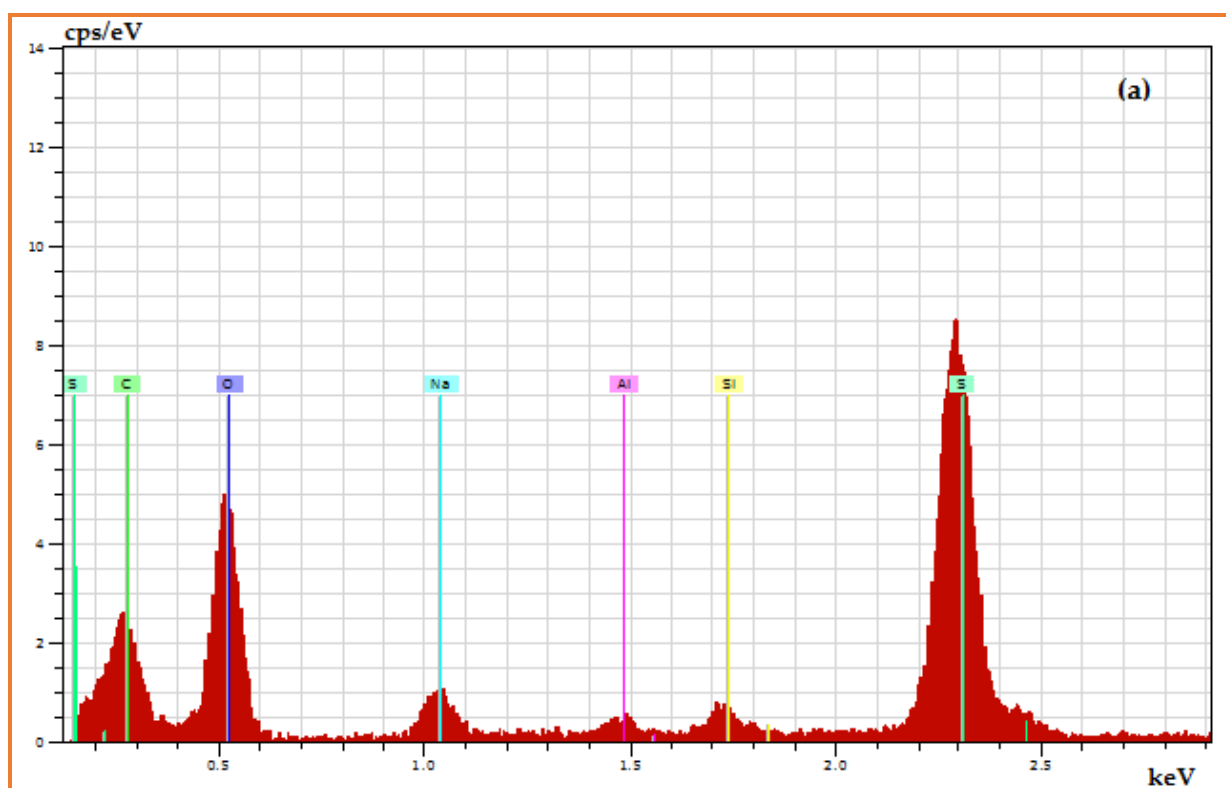


Figure 2. Lignin Biomass Yield at 120°C at different times.

The EDX analysis result is shown in Figure 3(a & b).



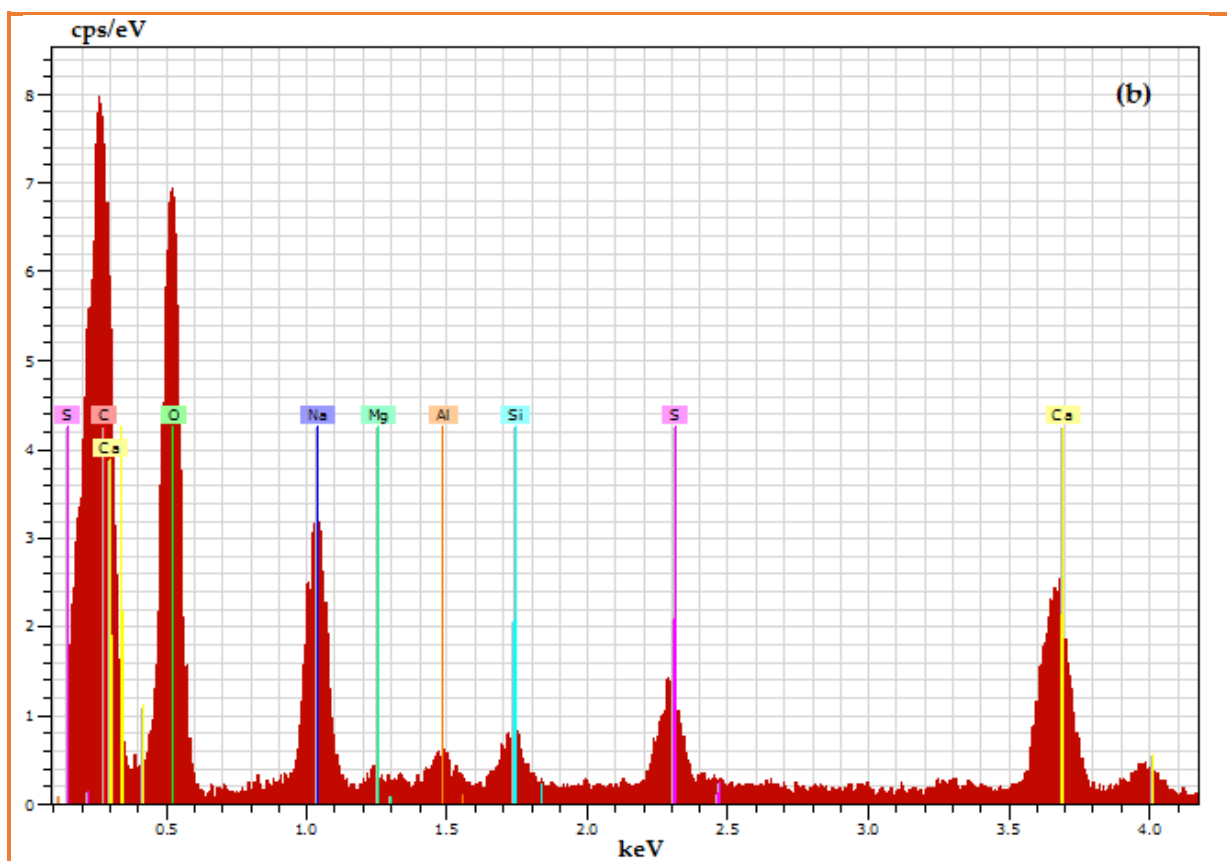


Figure 3. EDS Analyses of NTB and BTB.

However, as demonstrated in the EDS graphical representation of Figure 3, the bands centered at 2937 and 2843 cm^{-1} are primarily caused by CH stretching in aromatic methoxyl groups as well as in methyl and methylene groups on side chains. The entire lignin's bandwidth, which spans the range of 3410 to 3460 cm^{-1} , results from the hydroxyl groups in phenolic and aliphatic structures. Peaks at 2917 and 2847 cm^{-1} indicate the lignins of flax, jute, and hemp, appearing as strong bands in this region. These peaks are due to aliphatic methylene groups that have undergone CH-stretching, facilitated by fatty acids present in lignin preparations, which are clearly shown here. Weak to medium bands from unconjugated carbonyl/carboxyl stretching can be observed between 1705 and 1720 cm^{-1} . A shoulder at 1680 cm^{-1} likely results from conjugated carbonyl-carboxyl stretching. The band at 1705 cm^{-1} broadens in oxidized lignins (flax-ox), sulfur-free softwood lignin, and solvent-extracted hardwood lignin, compared to Kraft lignin and softwood lignosulfonates. Both protein contaminants and water, combined with lignin, can produce weak absorptions at 1650 cm^{-1} , leading to asymmetry and amplification of the more intense bands at 1705 and 1600 cm^{-1} .

Lignin can be seen in several substrates using SEM in Figure 4. SEM produces high-resolution images of a lignin sample surface using an electron beam. The surface morphology of the samples was examined using SEM and an electron microscope. The side chain length and acylating agent erosion both contributed to the surface layer at the same time.

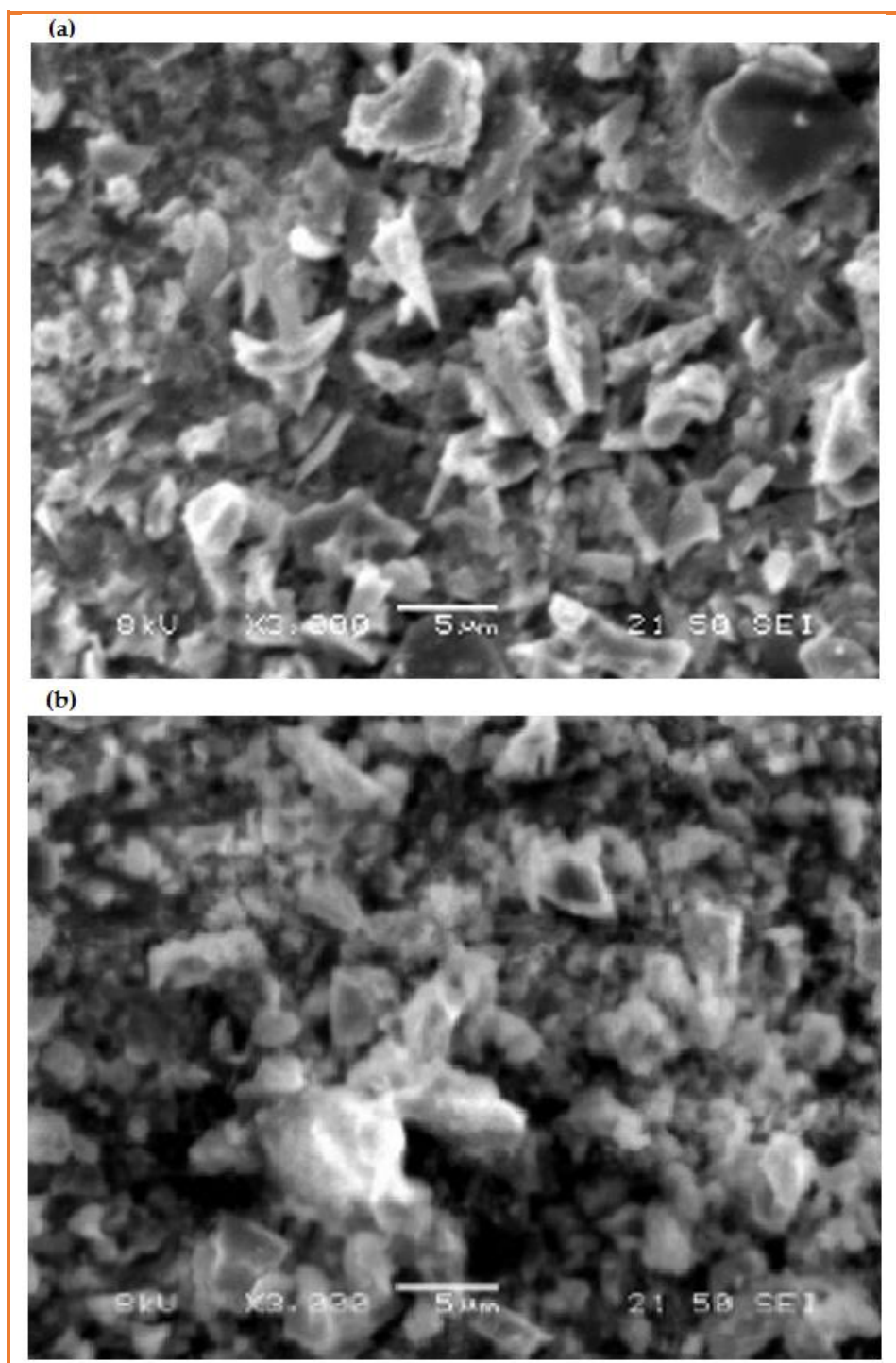


Figure 4. SEM analysis of (a) Neem tree bark and (b) Babul tree bark.

In this case, the SEM analysis may have measured the thickness of some components present in the NTB and BTB, such as cell walls, fibers, or other structures. The 5 μm indicates the average dimension of these features.

It is demonstrated in [Figure 5](#) and [6](#), the TGA analysis of BTB and NTB biomass extracted lignin. If the percentage change in sample mass decreases with an increase in temperature in a TGA curve plot (as observed in [Figure 5](#) and [6](#)), it typically indicates a process of mass loss or decomposition of the sample as the temperature rises.

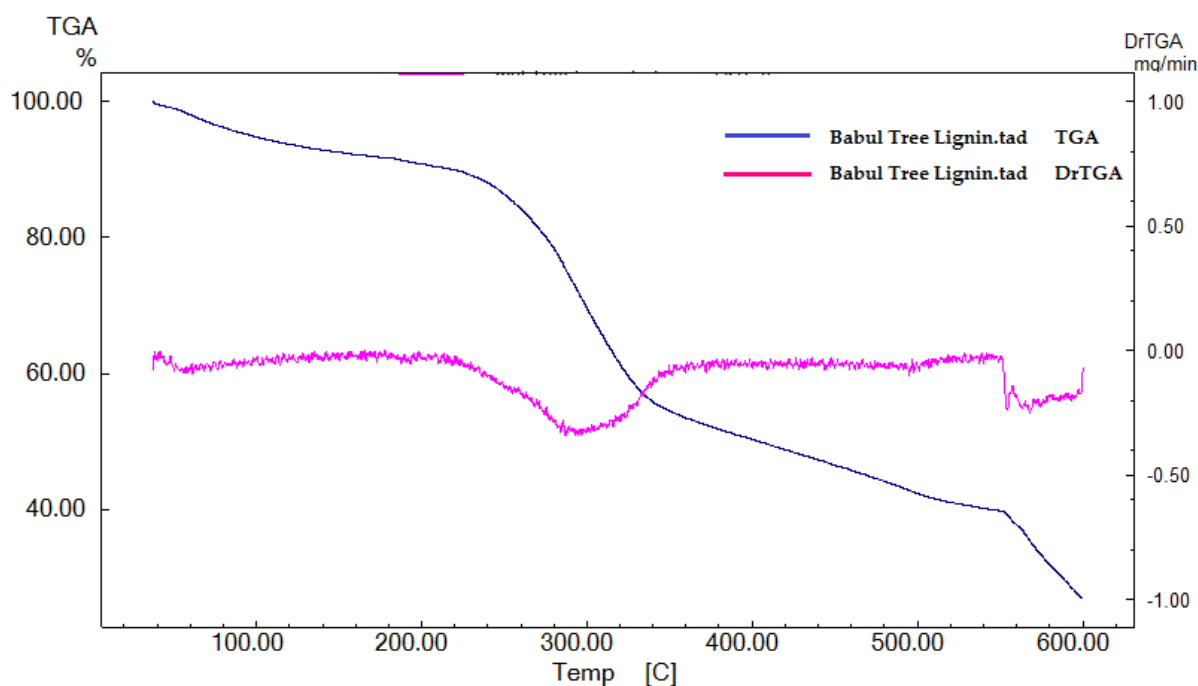


Figure 5. Analysis of the TGA of Babul tree bark.

Several possible scenarios can lead to a decrease in the percentage change in mass (Elshafie, Taha, Elhamamsy, Moustafa, & Elazab, 2020; Sebio-Punal, Naya, López-Beceiro, Tarrío-Saavedra, & Artiaga, 2012). Firstly, dehydration occurs because many materials, especially hydrated compounds like minerals or certain organic substances, contain water molecules as part of their structure. As the temperature increases, these materials can undergo dehydration, resulting in the loss of water molecules. This process leads to a decrease in the sample's mass, and the TGA curve will show a downward trend in the percentage change in mass as the temperature increases. Some materials may contain volatile components (low-boiling-point substances) that are weakly bound to the sample; as the temperature increases, these volatile components can desorb or evaporate from the sample, leading to a mass loss. The TGA curve will exhibit a decreasing trend in the percentage change in mass. Decomposition or degradation causes many organic and inorganic compounds to undergo thermal decomposition or degradation when exposed to increasing temperatures. During these processes, complex molecules break down into simpler products, often releasing gases, vapors, or volatiles. The loss of these decomposition products causes a decrease in the sample's mass over time, leading to a decreasing trend in the TGA curve, as shown in Figure 6. Some materials may undergo sublimation, transitioning directly from a solid to a gaseous state without passing through a liquid phase. This sublimation process leads to a mass loss and results in a decreasing percentage change in mass in the TGA curve as the temperature increases.

In TGA analysis, "DrTGA", as shown in Figure 5, likely refers to the derivative TGA curve. DrTGA is derived from the TGA data and provides information about the rate of weight loss or gain as a function of temperature or time. The DrTGA of BTB is nearly -0.25 mg/min at approximately constant (60%) TGA percentage at varying temperature (Figure 5). A negative DrTGA above implies a weight gain rather than weight loss as a function of temperature or time. This means that the BTB sample is gaining mass during the thermal analysis process. A negative DrTGA curve could be indicative of various components from the surrounding environment. It might also suggest that the sample undergoes a chemical reaction that leads to a net increase in mass.

It's important to interpret TGA data in conjunction with other complementary techniques to gain a comprehensive understanding of the thermal behavior of the sample. Differential Scanning Calorimetry (DSC) is often used alongside TGA to study the associated heat flow during thermal events, providing additional insights into the sample's behavior during temperature changes. A 10% weight loss in TGA (as shown in Figure 6) means that

10% of the original mass of the NTB lignin has been lost due to processes such as decomposition, evaporation, or other thermal transformations.

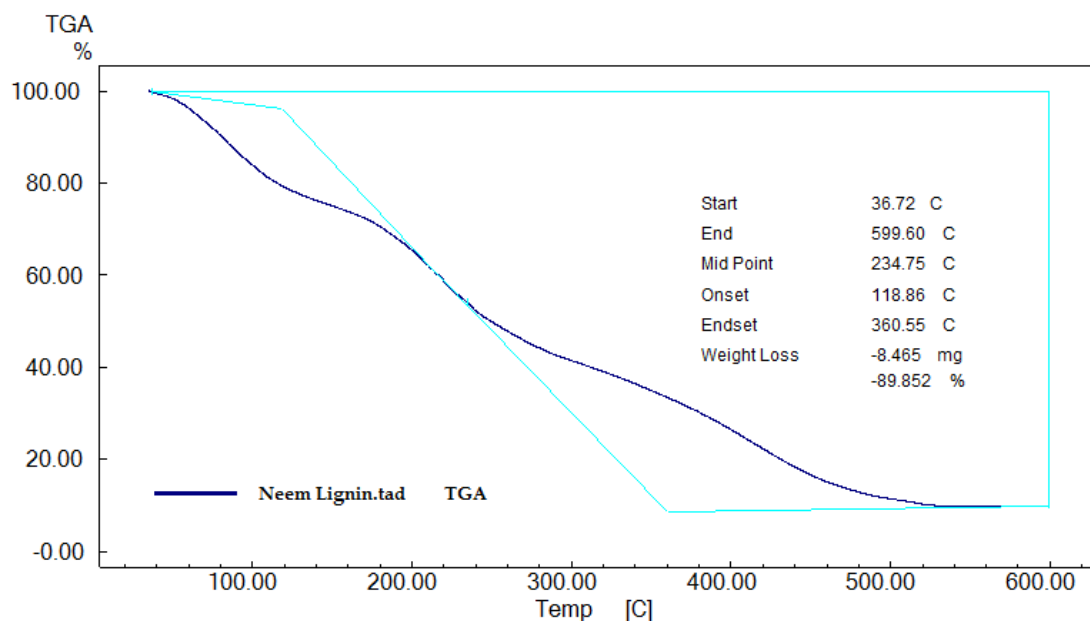


Figure 6. Analysis of the TGA of neem tree bark.

Interpreting TGA curves (Figure 5 and 6) requires expertise and knowledge of the material being analyzed. Additionally, it is essential to consider the experimental conditions, such as the heating rate, atmosphere, and sample size, as they can influence the observed thermal behavior. The start, onset, end, and endset temperatures of the TGA of NTB and the % weight loss (90%) are carefully marked using a light blue color shown in Figure 6.

In the TGA curve (Elshafie et al., 2020; Watkins et al., 2015), the terms ‘start’, ‘end’, ‘midpoint’, ‘onset’, and ‘endset temperatures’ refer to specific temperature points that mark significant events during the thermal analysis of the NTB lignin sample. These points provide valuable information about the sample’s thermal behavior and its decomposition or weight loss processes.

The ‘Start Temperature’ is the initial temperature at which the TGA analysis begins. It marks the point where the controlled temperature program starts to affect the sample. The ‘End Temperature’ is the final temperature of the TGA analysis. It marks the point where the controlled temperature program ends, and the analysis is complete. The ‘Midpoint Temperature’ is the average temperature between the start and end temperatures. It provides an approximate center temperature of the TGA curve, which can be useful for comparing different analyses or obtaining a representative temperature value. The ‘Onset Temperature’ is the temperature at which the thermal decomposition or weight loss process begins to occur significantly. It is the point where the TGA curve shows an initial deviation from the baseline, indicating the start of the decomposition process. Lastly, the ‘Endset Temperature’ is the temperature at which the thermal decomposition or weight loss process ends or becomes negligible. It is the point where the TGA curve returns to the baseline after the completion of the decomposition process.

5. CONCLUSION

The present investigation provides insights into lignin extraction from NTB and BTB biomass resources. Additionally, lignin extracted from BTB biomass demonstrated higher yields compared to NTB lignin. It was concluded that lignocellulosic feedstock is influenced by variables such as time and temperature during the extraction process. BTB, after 5 hours, achieved a lignin yield of 15%, whereas NTB yielded 14% less lignin. It was previously determined that acids and alkalis can be utilized in the extraction process. The extracted material was characterized using EDS, SEM, and TGA. The TGA temperature points facilitated understanding the kinetics and mechanisms of

thermal events occurring in the samples, providing valuable information for materials characterization and analysis. Although alkaline pretreatment for lignin recovery from biomass is more advantageous for commercial and domestic applications as a polymeric material, lignin obtained through this method is also biodegradable and biocompatible, making it suitable for use in medicine, composite materials, and wood products. The alkaline treatment effectively removed lignin from both BTB and NTB biomass according to instrumental analysis findings. However, a small portion of sugar impurities remains in biomass-derived lignin, which can be removed through acid treatment.

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Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

REFERENCES

- Ab Rahim, A. H., Man, Z., Sarwono, A., Wan Hamzah, W. S., Yunus, N. M., & Wilfred, C. D. (2018). Extraction and comparative analysis of lignin extract from alkali and ionic liquid pretreatment. *Journal of Physics: Conference Series*, 1123, 012052. <https://doi.org/10.1088/1742-6596/1123/1/012052>
- Akhter, F., Soomro, S. A., Jamali, A. R., Chandio, Z. A., Siddique, M., & Ahmed, M. (2023). Rice husk ash as green and sustainable biomass waste for construction and renewable energy applications: A review. *Biomass Conversion and Biorefinery*, 13(6), 4639-4649. <https://doi.org/10.1007/s13399-021-01527-5>
- Akram, H. A., Imran, M., Javaid, A., Latif, S., Rizvi, N. B., Jesionowski, T., & Bilal, M. (2023). Pretreatment and catalytic conversion of lignocellulosic and algal biomass into biofuels by metal organic frameworks. *Molecular Catalysis*, 539, 112893. <https://doi.org/10.1016/j.mcat.2022.112893>
- Anu, Kumar, V., Singh, D., & Singh, B. (2023). A greener, mild, and efficient bioprocess for the pretreatment and saccharification of rice straw. *Biomass Conversion and Biorefinery*, 13(5), 4121-4133. <https://doi.org/10.1007/s13399-021-01450-9>
- Ardhiansyah, H., Kusumaningrum, M., Bahlawan, Z. A. S., Prasetyawan, H., Savanti, F., & Fauziyyah, H. A. (2024). Green pretreatment techniques for enhanced delignification of lignocellulosic biomass: A case study of biomass waste in Indonesia. *IOP Conference Series: Earth and Environmental Science*, 1381, 012034. <https://doi.org/10.1088/1755-1315/1381/1/012034>.
- ASTM. (2021). Standard test method for volatile matter content of activated carbon samples. In ASTM D5832-98(2021). In. West Conshohocken, PA, USA: ASTM International.
- ASTM International. (2018). *Standard test method for total ash content of activated carbon (D2866-11, reapproved 2018)*. West Conshohocken, Pennsylvania, USA: ASTM International.
- Capolupo, L., & Faraco, V. (2016). Green methods of lignocellulose pretreatment for biorefinery development. *Applied Microbiology and Biotechnology*, 100(22), 9451-9467. <https://doi.org/10.1007/s00253-016-7884-y>
- Den, W., Sharma, V. K., Lee, M., Nadadur, G., & Varma, R. S. (2018). Lignocellulosic biomass transformations via greener oxidative pretreatment processes: Access to energy and value-added chemicals. *Frontiers in Chemistry*, 6, 141. <https://doi.org/10.3389/fchem.2018.00141>
- Dharmaraja, J., Shobana, S., Arvindnarayan, S., Francis, R. R., Jeyakumar, R. B., Saratale, R. G., . . . Kumar, G. (2023). Lignocellulosic biomass conversion via greener pretreatment methods towards biorefinery applications. *Bioresource Technology*, 369, 128328. <https://doi.org/10.1016/j.biortech.2022.128328>
- Efetobor, U. J., Ikpeseni, S. C., & Sada, S. O. (2022). Determination of proximate, ultimate and structural properties of elephant grass as biomass material for bio-oil production. *Journal of Applied Sciences and Environmental Management*, 26(12), 1903-1907. <https://doi.org/10.4314/jasem.v26i12.3>

- Elshafie, M., Taha, M. G., Elhamamsy, S. M., Moustafa, Y., & Elazab, W. I. M. (2020). Thermal analysis of the prepared lignin/graphene oxide/polyurethane composite. *Egyptian Journal of Petroleum*, 29(2), 195–201. <https://doi.org/10.1016/j.ejpe.2020.04.001>
- Erdocia, X., Hernández-Ramos, F., Morales, A., Izaguirre, N., de Hoyos-Martínez, P. L., & Labidi, J. (2021). Lignin extraction and isolation methods. In H. Santos & P. Figueiredo (Eds.), *Lignin-based materials for biomedical applications: Preparation, characterization, and implementation*. In (pp. 61–104). Elsevier. <https://doi.org/10.1016/B978-0-12-820303-3.00004-7>
- Garedew, M., Lin, F., Song, B., DeWinter, T. M., Jackson, J. E., Saffron, C. M., . . . Anastas, P. T. (2020). Greener routes to biomass waste valorization: Lignin transformation through electrocatalysis for renewable chemicals and fuels production. *ChemSusChem*, 13(17), 4214–4237. <https://doi.org/10.1002/cssc.202000987>
- Grzybek, J., Sepperer, T., Petutschnigg, A., & Schnabel, T. (2021). Organosolv lignin from European tree bark: Influence of bark pretreatment. *Materials*, 14(24), 7774. <https://doi.org/10.3390/ma14247774>
- Guan, J., Khan, A., Zhang, Y., Zhou, Y., Li, M. M.-J., Patria, R. D., & Leu, S.-Y. (2025). Strategic design principles for greener biorefineries: A substrate–process matrix emphasizing complete lignocellulose utilization from various biomass feedstocks. *Green Chemistry*, 27(38), 11581–11606. <https://doi.org/10.1039/D5GC02627J>
- Gunny, A. A. N., & Arbain, D. (2013). Ionic liquids: Green solvent for pretreatment of lignocellulosic biomass. *Advanced Materials Research*, 701, 399–402. <https://doi.org/10.4028/www.scientific.net/AMR.701.399>
- Gunny, A. A. N., Arbain, D., & Jamal, P. (2017). Effect of structural changes of lignocelluloses material upon pre-treatment using green solvents. *AIP Conference Proceedings*, 1835(1), 020022. <https://doi.org/10.1063/1.4981844>
- Hu, G., Heitmann, J. A., & Rojas, O. J. (2008). Feedstock pretreatment strategies for producing ethanol from wood, bark, and forest residues. *BioResources*, 3(1), 270–294. <https://doi.org/10.15376/biores.3.1.270-294>
- Karnaouri, A., Rova, U., & Christakopoulos, P. (2016). Effect of different pretreatment methods on birch outer bark: New biorefinery routes. *Molecules*, 21(4), 427. <https://doi.org/10.3390/molecules21040427>
- Kumar, P., Kermanshahi-pour, A., Brar, S. K., & Brooks, M. S.-L. (2021). Conversion of lignocellulosic biomass to reducing sugars in high pressure and supercritical fluids: Greener alternative for biorefining of renewables. *Advanced Sustainable Systems*, 5(4), 2000275. <https://doi.org/10.1002/adsu.202000275>
- López-Linares, J. C., García-Cubero, M. T., Lucas, S., González-Benito, G., & Coca, M. (2019). Microwave assisted hydrothermal as greener pretreatment of brewer's spent grains for biobutanol production. *Chemical Engineering Journal*, 368, 1045–1055. <https://doi.org/10.1016/j.cej.2019.03.032>
- Morán-Aguilar, M. G., Costa-Trigo, I., Aguilar-Uscanga, M. G., Paz, A., & Domínguez, J. M. (2024). Development of sustainable sugarcane bagasse biorefinery using a greener deep eutectic solvent from hemicellulose-derived acids. *Biomass and Bioenergy*, 186, 107256. <https://doi.org/10.1016/j.biombioe.2024.107256>
- Negro, M. J., Álvarez, C., Doménech, P., Iglesias, R., & Ballesteros, I. (2020). Sugars production from municipal forestry and greening wastes pretreated by an integrated steam explosion-based process. *Energies*, 13(17), 4432. <https://doi.org/10.3390/en13174432>
- Neiva, D. M., Rencoret, J., Marques, G., Gutiérrez, A., Gominho, J., Pereira, H., & Del Río, J. C. (2020). Lignin from tree barks: Chemical structure and valorization. *ChemSusChem*, 13(17), 4537–4547. <https://doi.org/10.1002/cssc.202000431>
- Norrrahim, M. N. F., Huzaifah, M. R. M., Farid, M. A. A., Shazleen, S. S., Misenan, M. S. M., Yasim-Anuar, T. A. T., . . . Jenol, M. A. (2021). Greener pretreatment approaches for the valorisation of natural fibre biomass into bioproducts. *Polymers*, 13(17), 2971. <https://doi.org/10.3390/polym13172971>
- Roy, R., Rahman, M. S., & Raynie, D. E. (2020). Recent advances of greener pretreatment technologies of lignocellulose. *Current Research in Green and Sustainable Chemistry*, 3, 100035. <https://doi.org/10.1016/j.crgsc.2020.100035>
- Sebio-Punal, T., Naya, S., López-Beceiro, J., Tarrío-Saavedra, J., & Artiaga, R. (2012). Thermogravimetric analysis of wood, holocellulose, and lignin from five wood species. *Journal of Thermal Analysis and Calorimetry*, 109(3), 1163–1167. <https://doi.org/10.1007/s10973-011-2133-1>

- Siddique, M., Mengal, A. N., Khan, S., Khan, L. A., & Kakar, E. K. (2023). Pretreatment of lignocellulosic biomass conversion into biofuel and biochemical: A comprehensive review. *MOJ Biology and Medicine*, 8(1), 39-43. <https://doi.org/10.15406/mojbm.2023.08.00181>
- Singh, Y. D., Mahanta, P., & Bora, U. (2017). Comprehensive characterization of lignocellulosic biomass through proximate, ultimate and compositional analysis for bioenergy production. *Renewable Energy*, 103, 490-500. <https://doi.org/10.1016/j.renene.2016.11.039>
- Stark, N. M., Yelle, D. J., & Agarwal, U. P. (2016). Techniques for characterizing lignin. In O. Faruk, M. Sain, M. Deans, D. Jackson, P. Gane, S. Li, & M. Rogers (Eds.), *Lignin in Polymer Composites*. In (pp. 49–66). Elsevier Inc: William Andrew Publications. <https://doi.org/10.1016/B978-0-323-35565-0.00004-7>
- Sun, S.-C., Xu, Y., Ma, C.-Y., Zhang, C., Zuo, C., Sun, D., . . . Yuan, T.-Q. (2023). Green and efficient fractionation of bamboo biomass via synergistic hydrothermal-alkaline deep eutectic solvents pretreatment: Valorization of carbohydrates. *Renewable Energy*, 217, 119175. <https://doi.org/10.1016/j.renene.2023.119175>
- Tao, Y., Li, S., Li, P., & Wu, Q. (2016). Thermogravimetric analyses (TGA) of lignins isolated from the residue of corn stover bioethanol (CSB) production. *Holzforschung*, 70(12), 1175-1182. <https://doi.org/10.1515/hf-2016-0022>
- Verma, P., Kumar, A., Kaushik, R., Sirohi, R., & Lai, C. W. (2025). Comparative analysis of green pretreatment strategies for optimized hemicellulose production from cauliflower (*Brassica oleracea* var. botrytis) waste: Beyond waste. *Journal of Environmental Chemical Engineering*, 13(6), 119464. <https://doi.org/10.1016/j.jece.2025.119464>
- Wang, N., Xu, A., Liu, K., Zhao, Z., Li, H., & Gao, X. (2024). Performance of green solvents in microwave-assisted pretreatment of lignocellulose. *Chemical Engineering Journal*, 482, 148786. <https://doi.org/10.1016/j.cej.2024.148786>
- Wardani, N. I., Samkumpim, T., Alahmad, W., King, A. W., Varanusupakul, P., Shishov, A., . . . Zain, N. N. M. (2024). Recent cutting-edge approaches to the integration of solid-liquid extraction with deep eutectic solvents: Toward a greener procedure for biomass valorization. *Advances in Sample Preparation*, 10, 100113. <https://doi.org/10.1016/j.sampre.2024.100113>
- Watkins, D., Nuruddin, M., Hosur, M., Tcherbi-Narteh, A., & Jeelani, S. (2015). Extraction and characterization of lignin from different biomass resources. *Journal of Materials Research and Technology*, 4(1), 26-32. <https://doi.org/10.1016/j.jmrt.2014.10.009>
- Yang, E., Chon, K., Kim, K.-Y., Le, G. T. H., Nguyen, H. Y., Le, T. T. Q., . . . Chae, K.-J. (2023). Pretreatments of lignocellulosic and algal biomasses for sustainable biohydrogen production: Recent progress, carbon neutrality, and circular economy. *Bioresource Technology*, 369, 128380. <https://doi.org/10.1016/j.biortech.2022.128380>
- Yiin, C. L., Quitain, A. T., Yusup, S., Uemura, Y., Sasaki, M., & Kida, T. (2018). Sustainable green pretreatment approach to biomass-to-energy conversion using natural hydro-low-transition-temperature mixtures. *Bioresource Technology*, 261, 361-369. <https://doi.org/10.1016/j.biortech.2018.04.039>
- Yimtrakarn, T., Kaveevivitchai, W., Lee, W.-C., & Lerkkasemsan, N. (2022). Study of lignin extracted from rubberwood using microwave assisted technology for fuel additive. *Polymers*, 14(4), 814. <https://doi.org/10.3390/polym14040814>
- Zhang, Z., Rackemann, D. W., Doherty, W. O. S., & O'Hara, I. M. (2013). Glycerol carbonate as green solvent for pretreatment of sugarcane bagasse. *Biotechnology for Biofuels*, 6(1), 153. <https://doi.org/10.1186/1754-6834-6-153>

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