

Study of the Effect of Time on Delignification of Young Bamboo Root for Biofuel Production

Yirakpoa Patience Nwambo*, Ipeghan Jonathan Otaraku

Department of Chemical Engineering, University of Port Harcourt, Port Harcourt, Nigeria

*Corresponding author

DOI: <https://doi.org/10.51244/IJRSI.2025.12040028>

Received: 22 March 2025; Accepted: 28 March 2025; Published: 02 May 2025

ABSTRACT

Due to fossil fuel and product issues, alternative energy and raw materials are being studied and created. The impact of time on the delignification of young bamboo root to improve its energy potential utilizing chemical pretreatment (alkali pretreatment) is examined here. Pretreated young bamboo roots were measured for moisture content. Sodium hydroxide (NaOH) pretreated the lignin, and hydrogen chloride (HCl) acid increased precipitation and yield %. Four samples (A, B, C, and D) were pretreated at constant temperature of 100°C for 30, 60, 90, and 120 minutes. Bamboo root mass was 15g, and NaOH solution concentration was 4mol/l. 0.5mol/l HCl acid was added to the filtrate until pH dropped to 2.0 to increase lignin precipitation before filtration. Acidified water pH=2.0 was used to wash and dry the lignin at specified temperature. Samples had A = 13.679g, B = 13.357g, C = 12.996g, and D = 12.221g after cellulose rich materials.. Lignin yields were 7.6%, 8.1%, 8.9%, and 9.7% for samples A=1.134g, B=1.215g, C=1.341g, and D=1.456g. Mature bamboo has more lignin than young bamboo. A yield within this range suggests that the delignification procedure is effective, but structural changes in lignin or residual lignin in treated bamboo may need to be analyzed to optimize it. This result suggests that optimizing the delignification duration is crucial for maximizing biofuel yield.

Keywords: Energy, young bamboo, time, delignification, chemical pretreatment.

INTRODUCTION

The rising demand for clean and renewable energy sources has led to significant research into biomass utilization. Bamboo is a fast growing and highly renewable resource, abundant in nature and with diverse applications in construction, paper production, textiles, and bioenergy. Nonetheless, its elevated lignin content presents a difficulty for its effective conversion into biofuels and other energy products. Delignification, the removal of lignin from lignocellulosic biomass, is essential for improving the usability of bamboo for various applications, including biofuel production, pulping, and chemical processing.

This study examines the impact of time on the delignification of young bamboo root to enhance its viability as an energy source. The effectiveness of delignification is influenced by several factors, including temperature, chemical agents, and reaction time. Among these, time plays a critical role in determining the extent of lignin removal and the preservation of cellulose structure. While extensive studies have been conducted on the delignification of mature bamboo, limited research has explored the delignification kinetics of young bamboo roots. Understanding how time affects the delignification process in young bamboo roots can provide valuable insights into optimizing industrial processes and improving efficiency. This research seeks to ascertain the ideal delignification duration that maximizes the energy potential of bamboo root while minimizing material degradation, by examining the correlation between delignification time and the degree of lignin removal. Throughout the 20th century, fossil fuels—primarily coal, natural gas, and petroleum—were the primary energy sources for the majority of businesses. They continue to be the most significant feedstocks for energy production worldwide. "Fossil fuels currently dominate the global energy market, which is valued at approximately 1.5 trillion dollars" [3]. These sources are far less available now, though, and are no longer

thought to be sustainable. [9] estimated the duration of oil, coal, and gas reserves to be around 35, 107, and 37 years, respectively. Furthermore, because of greenhouse gas emissions, their combustion contributes to environmental problems including global warming [8]. As a result, the last 20 years have seen an increase in interest in finding environmentally friendly and sustainable energy sources, which has led to the development of fuels made from renewable feedstocks like biomass. Wood, agricultural, industrial, or residential leftovers, and energy crops from dedicated farming are the three main groups into which these feedstocks are typically separated. [1]

Acids, alkalis, and solvents can be used to chemically separate lignin and carbohydrates by promoting the selective solubilization of each component. Carbohydrates dissolve in acidic solutions; lignin degrades and dissolves in alkaline solutions [6]; and carbohydrates dissolve in solvent-based solutions, commonly referred to as organogold pretreatment [12]. Chemical procedures may offer benefits in terms of necessary time, scalability, and process control, but they might not be as selective as biological processes.

Purified enzymes or microorganisms, which generate a group of enzymes that function in concert, can be used for biological delignification. Fungi belonging to the Basidiomycetes family are the most commonly used microorganisms. However, lignin and its derivatives can also be broken down by bacteria from strains of *Pseudomonas*, *Flavobacteria*, *Xanthomonas*, *Bacillus*, *Aeromonas*, and *Cellulomonas*. By cultivating the microorganism in submerged, semisolid, or solid cultures, where enzymes like lignin peroxidase, xylanase, laccase, and manganese peroxidase (among others) carry out selective lignin degradation, biological lignin degradation can be carried out.

[13] maintained LCB1, LCD1, and LCN1 strains in pure cultures in their lab at 4°C on potato dextrose agar (PDA) slants. To create inoculums for fungal pretreatment, these fungi were cultivated on PDA plates for ten days at 28°C. In order to produce enzymes, the researchers cultivated strains of LCB1, LCD1, and LCN1. Samples underwent centrifugation and analysis for Filter Paper Activity (FPase) and xylanase activity levels.

Enzymes like Lac, LiP, and MnP play a crucial role in lignin degradation by breaking it down in the extracellular space and were measured using an enzyme activity assay kit. The strains LCB1, LCD1, and LCN1 exhibited similar trends in enzymatic activity, with a preference for hemicellulose degradation. Shorter fermentation periods are the result of the newly isolated LCB1 and LCN1 fungal strains' rapid production of ligninolytic enzymes, the researchers discovered. Coculturing LCB1 and LCN1 together was more successful than employing either fungus alone at lowering the amount of lignin in bamboo, according to the physicochemical study of bamboo samples treated with both fungi. In particular, the coculture pretreatment preserved the cellulose content for further processing while primarily focusing on the destruction of lignin and hemicellulose. The coculture of LCB1 and LCN1 is a promising strategy for increasing the processing efficiency of lignocellulosic biomass because of this selective degradation.

[4] investigated the delignification saturation point in alkaline hydrogen peroxide (AHP) pretreatment of bamboo. The researchers found that increasing the hydrogen peroxide dosage from 0% to 2% improved lignin removal from 52.23% to approximately 70%. However, further increases in hydrogen peroxide concentration did not enhance lignin removal, indicating a saturation point. This suggests that beyond a certain threshold, additional delignification time or reagent does not significantly improve lignin extraction.

[11] studied the delignification of bamboo (*Bambusa procera acher*) using kraft pulping followed by oxygen-alkali. Delignification demonstrated that bamboo pulp could be effectively delignified to a low kappa number without significant loss in viscosity. This indicates the potential of bamboo as a viable feedstock for biofuel production.

[3] studied how green bamboo's hemicellulose was broken down by combinatorial pretreatments during hydrothermal pretreatment (HP). The hydrothermally pretreated bamboo was delignified using a deep eutectic solvent (DES) that contained lactic acid and choline chloride, which ultimately allowed for the enzymatic hydrolysis of the cellulose residue. Hemicellulose was removed by 88.6% after 35 minutes of hydrothermal treatment at 180 °C, whereas the yield and purity of xylo-oligosaccharides were 50.9% and 81.6%,

respectively. According to the findings, a promising method for overcoming lignocellulose's resistance to high value-added utilization is synergistic pretreatment.

[5] in a research on the delignification efficiency of various biomass types using a phenoxyethanol/acid mixture at 120 °C demonstrated that bamboo biomass could achieve up to 90% lignin removal. This highlights the potential of specific solvent mixtures in enhancing delignification efficiency.

[10] used a mixture of Celic C cellulase enzymes to evaluate the effects of pretreatment of *Dendrocalamus strictus* bamboo residues with different doses of alkali, hydrogen peroxide, and alkaline hydrogen peroxide on biomass digestibility in order to improve sugar recovery. Enzymatic hydrolysis data showed that untreated raw biomass had a 40% digestibility after 48 hours of incubation. The alkali-pretreated biomass had the maximum digestibility of 61% when loaded with 10% loaded with 0.5% w/v NaOH. Using 1% w/v H₂O₂ from biomass to pretreat bamboo shows a maximum digestibility of 75%. A maximum digestion efficiency of 83% was achieved when biomass loaded with 1% w/v NaOH-H₂O₂ was pretreated with alkaline hydrogen peroxide. The crystallinity index (CrI) analysis uncovered that in processed biomass. The results showed that alkaline hydrogen peroxide pretreatment techniques are an effective method for sugar recovery for the production of bioethanol and that bamboo waste biomass may be used as feedstock for biorefinery products.

The results of this study may enhance bioenergy technology, especially in areas with abundant bamboo resources. Furthermore, optimizing delignification conditions could facilitate the advancement of renewable energy alternatives and diminish dependence on fossil fuels.

MATERIALS AND METHODS

Materials

Reagents used

Chemicals like Sodium hydroxide (NaOH), Hydrochloric acid (HCl) and Distilled water were purchased from Joechem Chemical shop at Choba, Rivers State, Port Harcourt, where they sell chemicals

Biomass collection

Young Bamboo was obtained from Mile 3 Market, where they sell bamboo poles for building houses.

Standardization of NaOH

4 moles/L of Sodium hydroxide (NaOH) per 500ml (0.5litre) was prepared using the calculation below:

$$\text{Molarity} = \frac{\text{mass}}{\text{molarmass}} \times \frac{1}{\text{volume}} \quad (2.1)$$

Molecular weight of NaOH = 40g/mol

$$4\text{mol/l} = \frac{x}{40\text{g/mol}} \times \frac{1}{0.5\text{l}}$$

$$x = 4\text{mol/l} \times 40\text{g/mol} \times 0.5\text{l}$$

$$x = 80\text{g}$$

80g of NaOH was measured and dissolved in 420ml of distilled water to obtain 4mol/L solution of Sodium hydroxide (NaOH).

Pretreatment of young bamboo root

The obtained bamboo root was cut into slices of different sizes, washed with water to remove particles impurity and air dried in the sun for 24 hours. The size reduction of biomass was done with a kitchen blender.

The air-dried bamboo was finally dried in the oven at 100°C for 1 hour to remove moisture content.

The oven used was the one located in the chemical engineering laboratory situated at Choba campus in the university

Determination of % composition of moisture content

$$\% \text{ composition of moisture content} = \frac{m_B - m_{DB}}{m_B} \times 100 \quad \text{-----} \quad 2.2$$

Where m_B is mass of air-dried bamboo and m_{DB} is mass of dried bamboo with oven.



Figure 2.1 Mixture of Bamboo and NaOH solution before heating



Figure 2.2 Mixture of Bamboo and NaOH solution after heating

Delignification process (Alkali pretreatment)

Alkali pretreatment of bamboo root was conducted using the method proposed by Mousavioun et al., 2010 with slight modification. 15g of dried bamboo was loaded into 120ml of 4mol/l of NaOH solution in a 250ml round bottom flask. The ratio of bamboo to NaOH solution is 1:8. The mixture was then heated at 100°C using heating mantles for different samples (A, B, C and D) at different times 30mins, 60mins, 90mins and 120mins, while time to temperature remained constant ($t=35\text{mins}$). The mass of bamboo root, temperature and concentration of NaOH solution were kept constant.

The resulting black slurry of bamboo/NaOH mixture was poured into beaker and cooled to room temperature and then filtered using the vacuum pump to recover solid known as cellulose rich materials (CRM). The solid

fraction was washed with distilled water for 5 times to remove NaOH solution and dried in the oven at 100°C for 1hour and weighed.



Fig 2.3 Solid Mass Fraction (Cellulose Rich Material-CRM)



Fig 2.4 Extracted Lignin

The filtrate was acidified to pH of 2.0 with 0.5mol/L of HCl solution to enhance lignin precipitation. The precipitates obtained was filtered and washed with acidified water (pH 2.0) and dried in the oven at 100°C for 1hour and weighed.

percentage of lignin

$$\% \text{ of lignin} = \frac{m_{DLN}}{m_{DB}} \times 100\% \quad \text{-----} \quad 2.3$$

Where m_{DLN} is mass of dried lignin and m_{DB} is mass of dried bamboo

RESULTS AND DISCUSSIONS

Results

The NaOH help to solubilize and extract lignin from the young bamboo root by affecting acetyl group in hemicellulose and linkages of lignin-carbohydrate ester. During the process the strong polyring bonds of the C-C and C-O-C bonds crosslinking is attacked by the NaOH solution which resulted in bond-breaking between the intermolecular ester bonds crosslinking xylan hemicellulose and lignin which enable the extraction of lignin.

Percentage composition of moisture content

The percentage composition of moisture content was calculated using equation 2.2

$m_B = 142.147g$ and $m_{DB} = 68.960g$

% composition of moisture content = 51.50%

The moisture content of the bamboo root was approximately half weight of the total mass of the bamboo root.

Mass of solid fraction: cellulose rich materials (CRM)

Table 3.1: Mass of solid fraction A, B, C and D obtained after alkali pretreatment

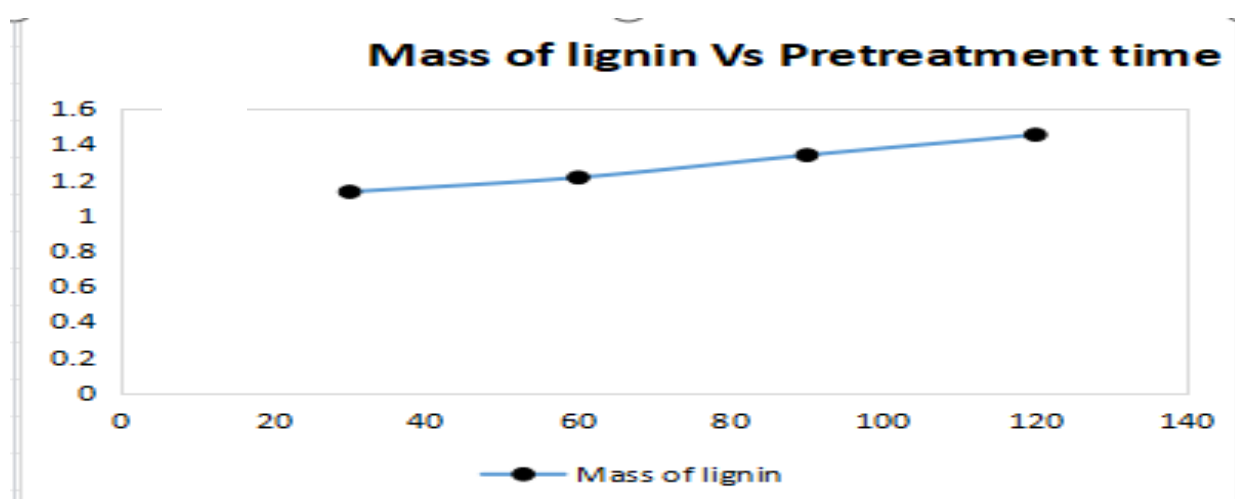
Samples	Time (mins)	Mass(CRM) (g)
A	30	13.679
B	60	13.357
C	90	12.996
D	120	12.221

Yield of lignin

The alkali pretreatment (NaOH solution) extracted lignin from young bamboo root and the lignin precipitates was enhanced with HCl. The yield percentage of lignin extracted and masses of each sample are as presented

Table 3. 2 showing yield percentage of lignin extracted and masses of each sample

Samples	Time (mins)	Mass of lignin	% of lignin (%)
A	30	1.134	7.56
B	60	1.215	8.10
C	90	1.341	8.94
D	120	1.456	9.71



DISCUSSION

The yield of 7.6% - 9.7% from younger bamboo exhibits a reduced lignin output, indicating that it inherently possesses less lignin than mature bamboo and woody biomass.

Mature bamboo possesses around 2–3 times greater lignin content, requiring more rigorous chemical or enzymatic treatment for delignification.

Delignification Efficiency: The percentage yield of lignin increased with increase in pretreatment time. In 30min, 60min, 90min and 120min, the quantity of removed lignin was 7.56%, 8.10%, 8.94% and 9.71% respectively of the initial mass. The graph shows that as pretreatment time increases, the mass of lignin yield increases. This is likely due to extraction of more lignin with increase in pretreatment time. Between 30 and 60 minutes, the mass decreased from 13.679 g to 13.357 g, indicating a little alteration. The drop persists from 60 to 90 minutes, culminating at 12.996g, indicating a consistent decline.

Nevertheless, between 90 and 120 minutes, the mass decreases markedly to 12.221 g, signifying an expedited decline in mass during the later phases. The reaction or loss mechanism may commence slowly, but it subsequently escalates over time.

This may indicate that external factors (e.g., temperature, reactant exhaustion, or phase transitions) affect the rate of mass diminution

Potential Clarification: The bulk of cellulose rich materials (CRM) diminish over time. The reduction in bulk is first slow but gets increasingly pronounced with time. If young bamboo initially comprises 10%–12% lignin, this technique eliminates approximately 63%–97% of it.

In pulping or bioethanol processes, efficient delignification generally eliminates 50%–90% of lignin, indicating that this operation is within an acceptable range. The reduction in mass over time indicates a process in which Cellulose rich material is being utilized, decomposed, or diminished. This may result from chemical reactions, evaporation, degradation, or adsorption, contingent upon the experimental environment.

Consequences for Implementations Biofuel Production: The reduction of lignin content, which obstructs chemical and enzymatic hydrolysis, increases the output of fermentable sugars, rendering this delignification process advantageous.

Composite Materials: To preserve structural integrity, a moderate reduction of lignin, as indicated in this work, is advantageous.

CONCLUSION

Identifying the appropriate delignification time is essential for maximizing efficiency in renewable energy applications involving bamboo biomass. The delignification results demonstrates a rather effective extraction of lignin from young bamboo, rendering it equivalent to certain agricultural leftovers and more amenable to processing than mature bamboo or hardwoods, it is recommended to extend the delignification time for optimisation tactics to enhance delignification yield.

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