

# Delignification of Different Lignocellulosic Biomass Using Hydrogen Peroxide and Acetic Acid (HPAC)

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**Abstract:** Lignocellulosic biomass will soon become the key source of feedstock for bioenergy to combat the impact of global warming and the depletion of fossil fuel resources. Lignin separation costs have been a key obstacle to bioenergy generation from lignocellulosic biomass. Pulp and paper, biofuel, and biomaterials sectors depend on wood delignification. This study examines delignification trends and advances, including standard and new methods. The study begins by explaining how delignification improves wood processability and value. It describes lignin's chemical structure and properties, highlighting its role in wood's mechanical and chemical qualities. Lignin from diverse lignocellulosic biomass sources is delignified using hydrogen peroxide and acetic acid in this study. A 1:1 hydrogen peroxide-acetic acid volume ratio was used after particle reduction. Experimental results show 96%, 89%, and 80% lignin removal efficiency for Ako, Mahogany, and mixed sawdust, respectively. A 30-minute reaction at 80°C yielded these results. The hydrogen peroxide-acetic acid mixture dignifies well and may be used in lignocellulosic biomass processing. This study also provides insights into optimizing delignification procedures and suggests approaches to improve the industrial use of lignocellulosic biomass. This study reveals that HPAC dignifies wood well. Understanding HPAC's role in the delignification of wood products could lead to developing an HPAC-based pretreatment technique that lowers the cost of biofuel generation from lignocellulosic biomass.

**Keywords:** Hydrogen Peroxide and Acetic Acid (HPAC); Hardwood and Softwood; Lignocellulosic Biomass; Mechanical and Chemical Properties; Value of Wood Material; Fossil Fuel Resources; Hydrogen Peroxide and Acetic Acid (HPAC).

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## 1. Introduction

The world's energy needs are mainly reliant on non-renewable fossil fuels, which are derivatives of crude oil; almost 90 % of the products are used for the generation of energy and mobility, among others. The problem of population explosion has led many developed and developing economies to increase their industrial activities, leading to rapid energy demands. It is certain to conclude that fossil fuels, which are the source of coal, natural gas, and oil, which are non-renewable energy, with time will

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be scarce as a result of depletion and inevitably exhausted and over-reliance on them at the expense of the climate for decades has led to a huge climatic change such as global warming due to emissions of greenhouse gas [1]. Therefore, it is important to explore every possibility of using an alternative energy source that is renewable, eco-friendly, and even more efficient than fossil fuels; thus, biofuel fermentation is key and integral. Renewable energy, such as biofuels, is a perfect replacement for fossil fuels and a worthy solution to economic challenges confronting the world and climate change's impact. Recently, more attention has been devoted to biofuel production and its use as an alternative that will help reduce greenhouse gas emissions [2]. The drastic rise in demand for ethanol use as a raw material for the production of other chemicals and as a fuel for Automobiles, economic and domestic activities like an energy source, preservatives, solvents for industrial processes, cleansing agents, and its unique role in fighting greenhouse gas emissions has led to the increase in demand and production [3].

Biofuel is derived from lignocellulose biomass of tree trimmings, grasses, and waste papers. These feedstocks contain lignocellulose, which includes lignin, hemicelluloses, and cellulose, which, when broken down, could release fermentable sugar for biofuel production [5]. Various treatment options are available for breaking down complex sugar into simple sugars; this can be done either through acidic hydrolysis at mild and high temperatures or through enzymatic hydrolysis, another important treatment option with limitations in the presence of lignin [6]. Our research interest explores the delignification pretreatment process for biofuel production from sawdust of different wood origin. As the case study, we will focus on the Ako tree's softwood sawdust and Mahogany's hardwood sawdust. Lignocellulose, the major component of the walls of the plant cell, is mostly made up of hemicellulose (about 20–40%), cellulose (about 40–60% of the total dry weight) and lignin (about 10–25%), [7].

The major obstacle in the hydrolysis reaction of cellulose from lignocellulose biomass to produce fermentable sugars lies in isolating it from lignin, those bonds with it making it difficult to expose to hydrolysis agent. In this research, we will study the effect of acid concentration on the lignocellulosic content of different sawdusts (softwood and hardwood) to yield fermentable sugar. Also, we will study the impact of cultured yeast (*Saccharomyces Cerevisiae*) concentration on the yield of biofuel; however, this paper will focus on the result from the delignification process, while the next paper will handle hydrolysis and fermentation part of the research. The research will also study the behaviours of wild yeast strains from rotten oranges in fermentation. Pretreatment is an expensive separation process responsible for nearly 33% of the overall cost of biofuel production; the economic process needs to be evaluated and dually improved if the system could be economically viable [8].

Lignocellulosic biomass, derived from diverse plant sources, represents a crucial renewable resource with significant potential for sustainable bio-based industries. Among its constituents, lignin, a complex aromatic polymer, poses challenges in biomass utilization due to its recalcitrant nature. Efficient delignification processes are essential to unlock the full potential of lignocellulosic biomass for various applications, including biofuels, bioproducts, and biochemicals. Delignification, the selective removal of lignin from biomass, is a pivotal step in biomass conversion processes. By reducing lignin content, the accessibility of cellulose and hemicellulose for enzymatic or chemical hydrolysis is improved, facilitating the production of value-added products. However, the efficacy of delignification methods varies depending on the characteristics of the lignocellulosic feedstock, such as species, composition, and structure [9].

In recent years, considerable efforts have been directed towards exploring novel delignification strategies capable of efficiently removing lignin from different lignocellulosic biomass sources. These approaches encompass various physical, chemical, and biological methods, each with advantages and limitations. Chemical delignification methods, in particular, have garnered significant attention due to their versatility and effectiveness in lignin removal. One such chemical delignification approach involves using a mixture of hydrogen peroxide and acetic acid. This method offers several advantages, including mild reaction conditions, high selectivity towards lignin, and minimal environmental impact. The combination of hydrogen peroxide and acetic acid has effectively disrupted lignin bonds, leading to substantial lignin removal while preserving the integrity of cellulose and hemicellulose.

Lignin components of hardwood (20-25%) and softwood (30-60%) are important to this research due to the dilution impact on the processes of fermentation and hydrolysis reactions. Thus, the procedure adopted in this research for the pretreatment process strictly addresses the economic needs and the release of microbial inhibitors that could reduce biofuel yield [10]. One of the major obstacles to the complete hydrolysis of lignocellulose biomass is the low availability of cellulose due to shielding by lignin. Hence, this problem can be overcome through an effective delignification reaction. This reaction was studied at a mild temperature of 80°C using Hydrogen Peroxide Acetic acid (HPAC) at a volume ratio of 1:1. This research aims to provide a comprehensive delignification process for various lignocellulosic biomass sources. Specifically, it focuses on the delignification of different lignocellulosic feedstocks using a hydrogen peroxide-acetic acid mixture as a delignifying agent. Through a critical analysis of recent research findings, this research seeks to elucidate the factors influencing delignification efficiency, assess the performance of different biomass sources, and identify opportunities for further optimization.

By synthesizing existing knowledge and highlighting recent advancements, this paper aims to contribute to developing sustainable and economically viable strategies for lignocellulosic biomass utilization. Understanding the intricacies of delignification processes is essential for advancing the bio-based economy and realizing the full potential of lignocellulosic biomass as a renewable feedstock for a wide range of industrial applications. Subsequently, the review examines traditional delignification methods, including kraft pulping, sulfite pulping, and soda pulping, highlighting their principles, advantages, and limitations. Special attention is given to these conventional techniques' environmental impact and sustainability considerations. Furthermore, the paper delves into recent advancements in delignification technologies, such as organosolv delignification, enzymatic delignification, and oxidative delignification. These emerging approaches offer promising alternatives to conventional methods, with potential benefits including reduced environmental footprint, higher selectivity, and improved product quality.

Moreover, the review discusses the influence of process parameters, such as temperature, pressure, and chemical additives, on the efficiency and selectivity of delignification processes. It also explores the synergistic effects of combined pretreatment strategies, such as delignification coupled with mechanical refining or fractionation, in optimizing the valorization of wood biomass. In addition, the paper addresses challenges and future directions in the field of wood delignification, including the development of cost-effective and sustainable delignification processes, elucidation of lignin structure-function relationships, and integration of delignification with downstream biorefinery processes. Overall, this review provides valuable insights into the current state of delignification research, offering researchers, engineers, and industry stakeholders a comprehensive understanding of the opportunities and challenges in this critical aspect of wood utilization and valorization.

This study explores the delignification of lignocellulosic biomass sourced from Ako sawdust, Mahogany sawdust, and a mixed sawdust sample, utilizing a novel delignification approach employing a mixture of hydrogen peroxide and acetic acid. The delignification process was carried out at a fixed temperature of 30°C with a reaction time of 30 minutes, following particle size reduction. A volumetric ratio of 1:1 of hydrogen peroxide to acetic acid was employed as the delignification reagent.

Experimental results demonstrate notable lignin removal efficiencies, with percentages reaching 96%, 89%, and 80% for Ako sawdust, Mahogany sawdust, and mixed sawdust, respectively. This remarkable delignification efficacy underscores the potential of the hydrogen peroxide-acetic acid mixture as an efficient delignification agent for various lignocellulosic biomass sources.

The study provides valuable insights into optimizing delignification parameters, including reagent concentration, temperature, and reaction time, to achieve maximum lignin removal while minimizing energy consumption and environmental impact. Furthermore, the investigation sheds light on the underlying mechanisms governing the delignification process and the structural alterations in lignocellulosic biomass following treatment with the hydrogen peroxide-acetic acid mixture. Overall, this research advances sustainable and efficient delignification methods, offering a promising avenue for valorizing lignocellulosic biomass for various industrial applications, including pulp and paper production, biofuel synthesis, and bio-composite manufacturing.

## 2. Literature Review

Delignification, the selective removal of lignin from lignocellulosic biomass, is crucial in various industries, including pulp and paper production, biofuel synthesis, and biorefinery processes. This paper aims to explore different delignification processes, encompassing traditional and emerging techniques, along with their principles, advantages, limitations, and recent advancements, and design an experiment for the delignification using HPAC. Future research directions may improve process efficiency, selectivity, and sustainability to facilitate the widespread adoption of delignification technologies in lignocellulosic biomass utilization. The general overview of the delignification process using traditional techniques is highlighted below:

### Kraft Delignification Process

- Principle: Utilizes an alkaline cooking liquor, typically a mixture of sodium hydroxide (NaOH) and sodium sulfide (Na<sub>2</sub>S), to dissolve lignin from the biomass.
- Advantages: High delignification efficiency, well-established industrial process.
- Limitations: Generates significant amounts of sulfurous pollutants, requires high energy input, and is non-selective towards lignin.
- Recent Advancements: Integration of kraft pulping with biorefinery processes for valorization of by-products.

### Sulfite Delignification Process

- Principle: Involves using acidic sulfite solutions (e.g., calcium, magnesium, or ammonium bisulfite) to solubilize lignin.

- Advantages: Moderate to high selectivity towards lignin, lower environmental impact compared to kraft pulping.
- Limitations: High chemical costs, formation of sulfurous pollutants, limited to certain types of wood.
- Recent Advancements: Development of sulfite pulping variants with enhanced selectivity and reduced environmental footprint.

#### Organosolv Delignification Process

- Principle: Utilizes organic solvents (e.g., ethanol, methanol, acetone) in combination with acids or alkalis to dissolve lignin.
- Advantages: Moderate to high selectivity, lower energy consumption compared to kraft pulping, and potential for valorization of lignin-rich liquor.
- Limitations: Higher chemical costs compared to conventional pulping methods limited scalability.
- Recent Advancements: Optimization of solvent systems, integration with biorefinery processes for co-production of value-added products.

#### Enzymatic Delignification

- Principle: Employs lignin-degrading enzymes (e.g., peroxidases, laccases) to degrade lignin selectively.
- Advantages: Mild reaction conditions, high selectivity, and potential for lignin valorization.
- Limitations: Slower reaction kinetics, dependence on enzyme stability and specificity, and higher costs.
- Recent Advancements: Engineering of enzymes for improved activity and stability, optimization of enzyme cocktails for specific biomass types [15].

#### Oxidative Delignification

- Principle: Involves using oxidative agents (e.g., oxygen, hydrogen peroxide) to degrade lignin selectively.
- Advantages: Mild reaction conditions, high selectivity, and potential integration with biorefinery processes.
- Limitations: Limited selectivity towards lignin, potential formation of toxic by-products.
- Recent Advancements: Developing catalytic systems for enhanced selectivity and efficiency and exploring novel oxidative agents.
- Combined Processes
- Principle: Integrating multiple delignification techniques (e.g., physical, chemical, enzymatic) enhances delignification efficiency and selectivity.
- Advantages: Synergistic effects lead to improved lignin removal and potential for co-production of value-added products.
- Limitations: Increased process complexity, higher capital and operational costs.
- Recent Advancements: Optimization of process parameters, development of integrated biorefinery concepts for holistic biomass valorization [12].

### 3. Chemical Composition of Lignocelluloses Biomass

Lignocellulose materials with high lignin content and ash composition are unfavourable for biofuel production. Lignin is a complex organic polymer found in the cell walls of plants, particularly in wood, where it plays a crucial role in providing structural support and rigidity. It is the second most abundant polymer on Earth, surpassed only by cellulose. Chemically, lignin is a heterogeneous polymer composed of phenylpropane units, primarily coniferyl, sinapyl, and coumaryl alcohols, which are interconnected by various types of linkages such as  $\beta$ -O-4,  $\beta$ -5, and  $\beta$ - $\beta$  bonds [13].

The chemical structure of lignin is characterized by its three-dimensional, amorphous nature, which contributes to its insolubility in water and most organic solvents. This structural complexity makes lignin resistant to enzyme degradation, rendering it a formidable barrier to biochemical processes such as cellulose hydrolysis.

Inwood, lignin acts as a binder, cementing together cellulose and hemicellulose fibres, imparting strength and rigidity to the cell wall structure. This reinforcement effect significantly influences the mechanical properties of wood, including its tensile strength, compressive strength, and resistance to bending and deformation. The presence of lignin also contributes to the hydrophobic nature of wood, making it less susceptible to moisture absorption and decay [14].

Moreover, lignin plays a crucial role in determining the chemical properties of wood, particularly its reactivity and susceptibility to various chemical processes. Lignin's aromatic structure and functional groups make it a target for chemical modification and utilization in various industrial processes, including pulping, biofuel production, and biorefinery processes.

Overall, lignin's chemical structure and properties are instrumental in shaping wood's mechanical and chemical characteristics, making it a pivotal component in utilizing and processing lignocellulosic biomass for various industrial applications.

### 3.1. Enzymatic Hydrolysis of Sawdust of Hardwood and Softwood

Zhang et al. [4] worked extensively on enzymatic hydrolysis, and they described the process as a medium-involving multistep reaction where insoluble cellulose is broken down at the solid-liquid interface. Malinen et al. [2] also confirmed that the solid-liquid interface reaction is via the synergetic action of endoglucanases and cellobiohydrolases.

Hydrolysis involving enzymes' flexibility with (Simultaneous Saccharification and Fermentation) SSF medium is significant. The simultaneous conversion of complex sugar to simple sugar and fermentation reaction can occur simultaneously in this process. However, the basic problem encountered is the deactivation of enzymes in the presence of lignin during the reaction.

### 3.2. HPAC Method of Delignification (Hydrogen Peroxide-Acetic acid Pretreatment)

Hydrogen peroxide-acetic acid pretreatment is a method used to remove lignin and enhance the accessibility of cellulose and hemicellulose in lignocellulosic biomass for downstream processes such as enzymatic hydrolysis and biofuel production. This pretreatment involves using a mixture of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and acetic acid (CH<sub>3</sub>COOH) under specific temperature and reaction time conditions. Saha [3], in their study of lignocellulosic materials using different techniques, including HPAC, concluded that an efficient and cost-effective process depends on a pretreatment system. Almeida et al. [14] also concluded that efficient removal of lignin will be key to minimizing the degradation of cellulose.

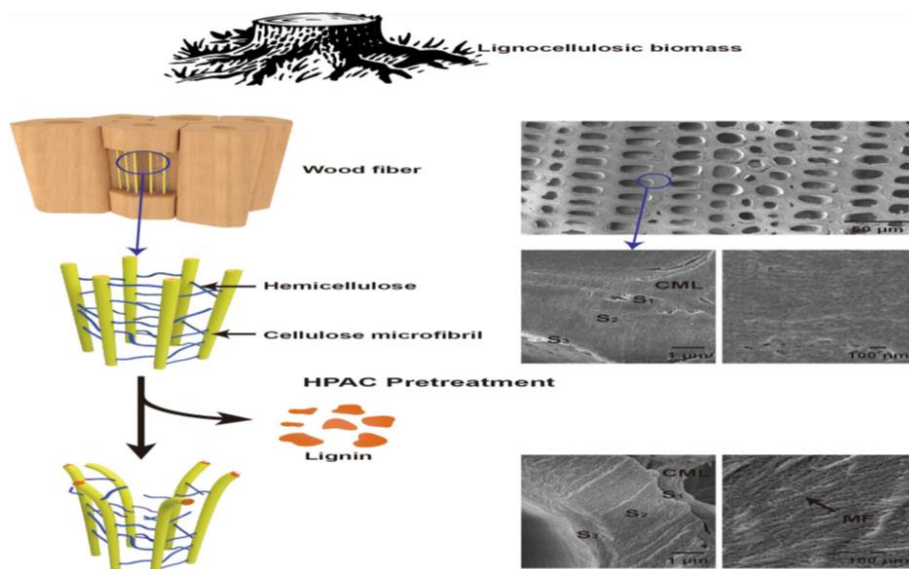
### 3.3. HPAC pretreatment Features

Ladisch et al. [5] developed a new process called the HPAC pretreatment method, depicted in Figure 1 below. They mixed hydrogen peroxide with acetic acid, which effectively removes lignin. They used lignocellulose biomass of oak wood, pine wood, and rice straw. Their study discussed pretreatment conditions, lignin removal efficiency, and cellulose and hemicellulose accessibility, as highlighted below.

Pretreatment Conditions: Lignocellulosic biomass is treated with hydrogen peroxide and acetic acid at specific ratios and concentrations. Pretreatment conditions, including temperature and reaction time, are crucial factors influencing the effectiveness of lignin removal and cellulose accessibility.

Lignin Removal: The hydrogen peroxide-acetic acid mixture disrupts the lignin structure in the biomass. Hydrogen peroxide helps in the oxidation of lignin, breaking down its complex chemical structure, while acetic acid facilitates the solubilization and removal of lignin fragments from the biomass matrix. This process effectively reduces the lignin content in the biomass and increases the exposure of cellulose and hemicelluloses.

Cellulose and Hemicellulose Accessibility: The removal of lignin and partial depolymerization of hemicelluloses during pretreatment lead to increased accessibility of cellulose and hemicellulose to enzymes during subsequent hydrolysis processes. This enhanced accessibility improves the efficiency of enzymatic hydrolysis, resulting in higher yields of fermentable sugars for biofuel production.



**Figure 1:** The concept of lignin removal using HPAC Pretreatment medium [16]

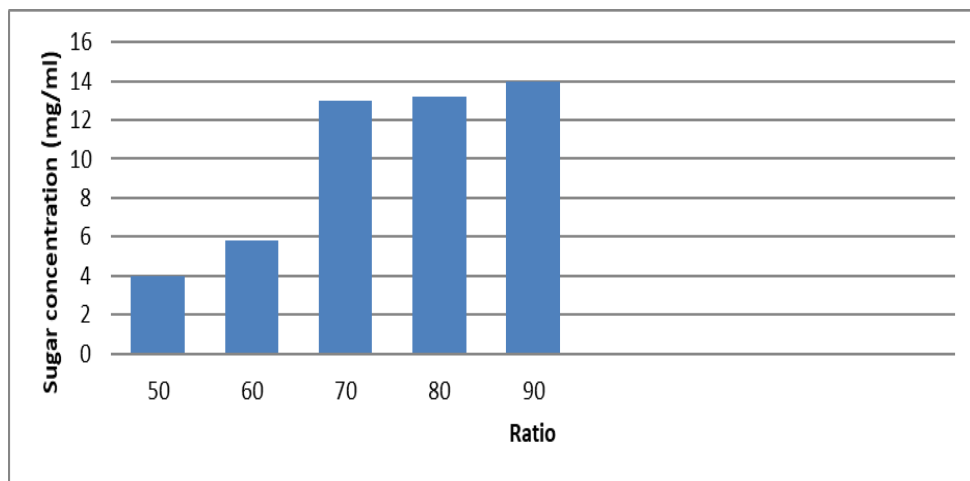
### 3.4. Optimal Conditions of HPAC Method of Pretreatment

Cui et al. [11] optimized the conditions for pretreatment by varying the ratio of hydrogen peroxide and acetic acid. They talked about inhibitor reduction and the process's economic and environmental considerations.

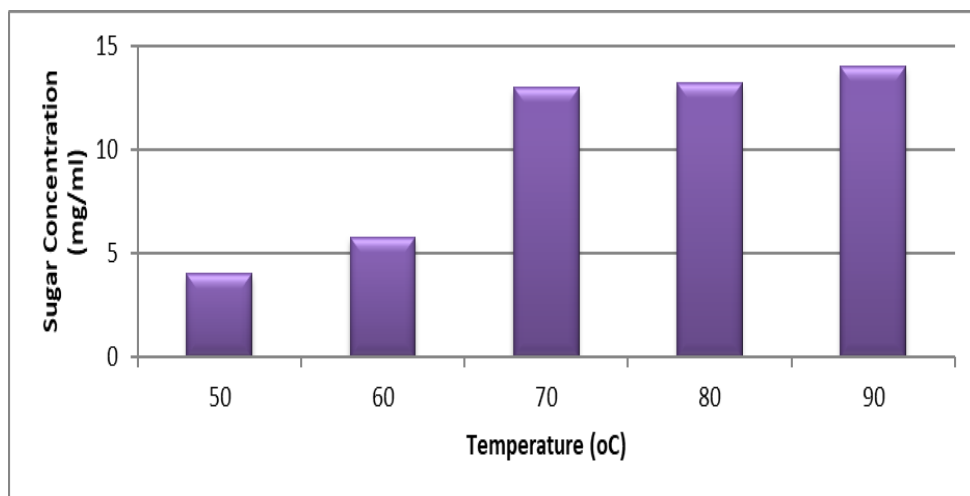
**Inhibitor Reduction:** Another benefit of hydrogen peroxide-acetic acid pretreatment is the reduction of inhibitory compounds that may hinder enzymatic hydrolysis and fermentation processes. By removing lignin and partially breaking down hemicellulose, the release of inhibitory compounds is minimized, improving the overall efficiency of biomass conversion.

**Economic and Environmental Considerations:** Hydrogen peroxide-acetic acid pretreatment is relatively mild compared to other pretreatment methods, such as dilute acid or steam explosion. This makes it an attractive option due to its lower energy consumption and reduced formation of inhibitory compounds. Additionally, using environmentally friendly reagents like hydrogen peroxide and acetic acid aligns with sustainability goals in biomass conversion processes.

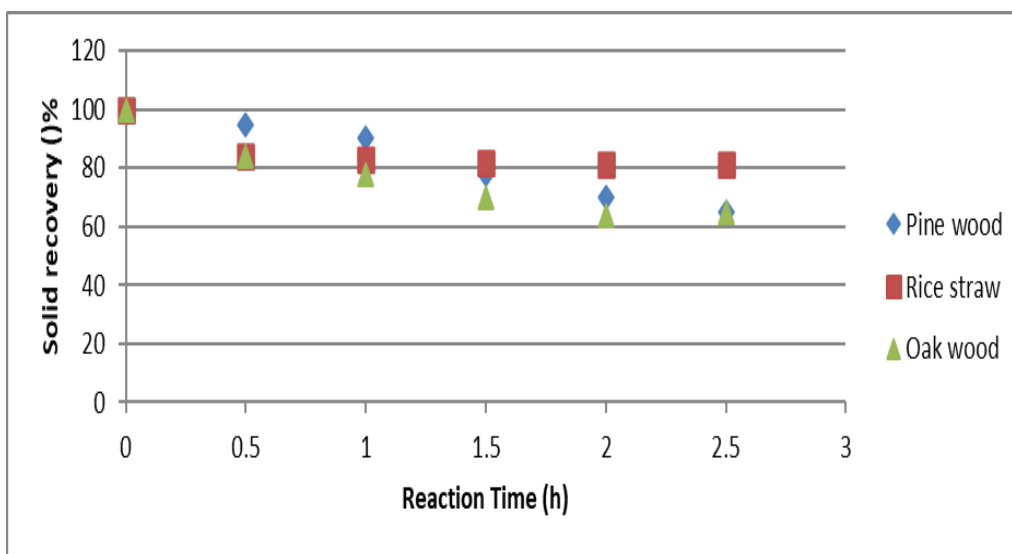
In conclusion, they stated that hydrogen peroxide-acetic acid pretreatment offers a promising approach to enhance the efficiency of lignocellulosic biomass conversion for biofuel production. Its ability to selectively remove lignin, improve cellulose accessibility, and reduce inhibitor formation makes it a valuable pretreatment method in developing sustainable biorefineries. However, optimization of pretreatment conditions and further research are needed to maximize their effectiveness and scalability in industrial applications (Figures 2 to 4).



**Figure 2:** Graphical illustration of the impact of hydrogen peroxide and acetic acid on lignin (a)



**Figure 3:** Graphical illustration of the impact of hydrogen peroxide and acetic acid on lignin (b)



**Figure 4:** Graphical illustration of the impact of hydrogen peroxide and acetic acid on lignin (c)

### 3.5. HPAC (hydrogen peroxide–acetic acid) pretreatment

Cui et al. [11] also worked on lignocellulose materials of Pinewood, Oakwood, and Rice straw using the HPAC process. However, they discovered that the basic disadvantage in their research work is the duration of the reaction of two hours before achieving meaningful delignification, meaning the reaction time is unnecessarily long; hence, there is a need to study ways to reduce the reaction time.

### 3.6. Enzymatic Hydrolysis and Ultrasonic Pretreatment

Mahmoudi et al. [7]: In their study of the conversion of pinewood into biofuel using the method of Ultrasonic Pretreatment followed by enzymatic hydrolysis, they described three processes:

- Pretreatment system with better digestibility of hemicellulose and cellulose in the lignocellulose biomass;
- A system that can improve the recovery of fermentable sugars via the pre-treated material and
- Fermentation system that can convert the sugars to ethanol in an efficient way.
- They argued that the real cause of the low sugar yield is the recalcitrance of cellulose related to lignin in pinewood.

### 3.7. Raw materials grinding

Shen et al. [8] described a process of grinding lignocellulose biomass for maximum impact of the hydrolysis reaction. They performed their experiment in a two-stage scheme. For the first stage, they passed lignocellulose material through a knife milling machine operating at about 15 rpm for five minutes. For the second stage, they passed the output of the first stage through a PM400 vario-planetary ball mill at 400 rpm for thirty minutes. They were able to obtain high penetration into the cell of the feedstock during the hydrolysis step. However, a lot of energy is required for the process.

Grinding lignocellulosic biomass is a mechanical pretreatment method aimed at increasing the surface area and accessibility of the biomass to chemical agents during delignification reactions. This process involves reducing the particle size of biomass feedstock through mechanical forces such as grinding, milling, or crushing. Here's an overview of grinding lignocellulosic biomass for maximum impact during delignification reactions:

**Increased Surface Area:** Grinding biomass into smaller particles increases its surface area-to-volume ratio. This increased surface area exposes more lignocellulosic components, including cellulose, hemicellulose, and lignin, to the chemical agents used in delignification, such as acids, alkalis, or enzymes. As a result, the delignification reaction can proceed more efficiently due to enhanced contact between the biomass and the delignifying agents.

**Improved Reactivity:** Smaller particle sizes resulting from grinding lead to improved reactivity of the biomass components. Cellulose and hemicellulose, primarily responsible for carbohydrate-derived products, become more accessible for hydrolysis or enzymatic digestion, resulting in higher yields of fermentable sugars. Additionally, the increased accessibility of lignin facilitates its removal during delignification, leading to higher lignin removal efficiencies.

**Reduced Mass Transfer Limitations:** Larger biomass particles may suffer from mass transfer limitations, where chemical agents penetrate only the outer layers of the biomass, leaving the inner regions untreated. Grinding reduces the diffusion path lengths

within the biomass particles, thereby reducing mass transfer limitations and ensuring a more uniform treatment of the biomass throughout the delignification process.

**Enhanced Reaction Kinetics:** Grinding lignocellulosic biomass can improve the kinetics of delignification reactions by reducing the time required for chemical agents to penetrate the biomass and react with lignin. This acceleration of reaction kinetics results in shorter processing times and increased throughput in industrial-scale delignification processes.

**Energy Requirements:** While grinding enhances the effectiveness of delignification reactions, it also incurs energy costs associated with mechanical processing. Careful consideration of energy inputs versus the benefits of increased delignification efficiency is necessary to optimize the overall process economics.

**Particle Size Optimization:** The effectiveness of grinding lignocellulosic biomass depends on achieving an optimal particle size distribution. Particle sizes that are too small may result in increased energy consumption during grinding and compaction issues during subsequent processing steps. Conversely, too large particles may not fully exploit the benefits of increased surface area and reactivity. Therefore, particle size optimization is crucial to maximize the impact of grinding on delignification reactions.

In conclusion, grinding lignocellulosic biomass is an effective pretreatment strategy to enhance the efficiency of delignification reactions. Grinding facilitates more thorough and rapid lignin removal by increasing surface area, improving reactivity, and reducing mass transfer limitations. This leads to higher yields of fermentable sugars and other valuable products from biomass feedstocks. However, careful optimization of particle size and consideration of energy requirements are essential to maximize the benefits of grinding while maintaining process sustainability and economics.

#### 4. Materials and Methods

In this study, lignocellulose biomass (sawdust) of particle size 2.00mm was used; Sample A: Hardwood sawdust (Mahogany), Sample B: Softwood sawdust (Ako), Sample C: Mixed Sawdust of Hardwood /Softwood, bio-digester, digital PH meter, High Powered Liquid Chromatography (HPLC), Spectrophotometer.

#### 5. Delignification Pretreatment process

Hydrogen peroxide was mixed with acetic acid to form Solution A in a ratio of 1:1 by volume, 10g of Sample A, B, and C were treated with 100 mL of Solution A separately and were heated to 80 °C for 150 minutes in a Water bath thermostatic heater, the mixture was filtered after that. Distilled water was used extensively to wash the residues for the pH to reach seven, and the residues were oven-dried at moderate temperature for 30 minutes. Figure 1 shows the lignocellulose biomass before and after pretreatment with HPAC

The reaction time was started after samples reached the temperature of 80oC. After Pretreatment with HPAC solution, the lignocellulose slurries were filtered through a 30-mL Kimax Gooch filtering mesh screen, and the water-soluble fraction was isolated from solid residue. The solid residue was weighed to measure the weight loss and determine the percentage of lignin removed.



(a) Lignocellulose biomass before pretreatment with HPAC





(b) Ako sawdust after pretreatment with HPAC



(c) Mahogany sawdust after pretreatment with HPAC

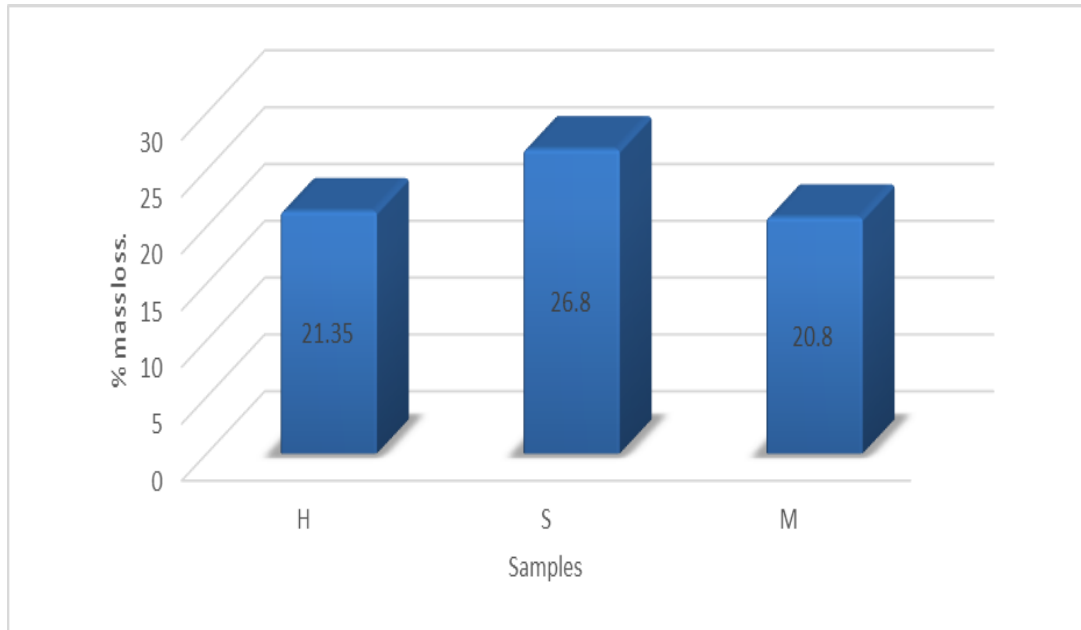


(d) mixed sawdust after pretreatment with HPAC

**Figure 5:** Lignocellulose biomass before and after pretreatment with HPAC (a, b, c, d)

## 6. Results from Delignification Reaction

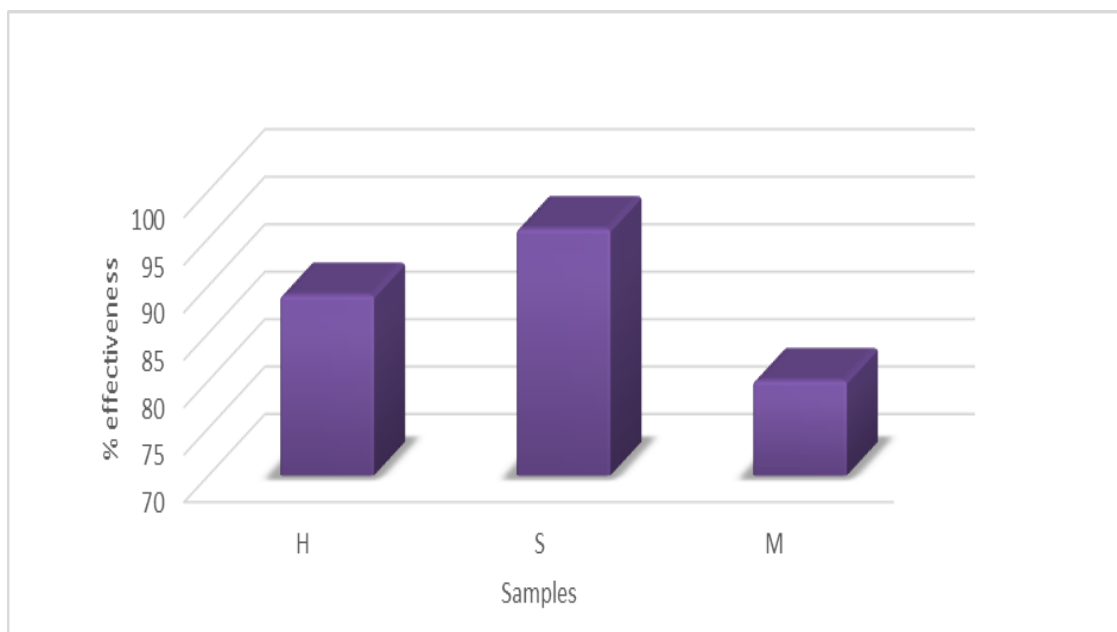
Figure 5 shows the percentage mass loss after delignification. It is obvious that the Hardwood sawdust of the Mahogany tree (H) lost 21.35 % of weight, representing 89.00% of lignin removed, the Softwood sawdust of the Ako tree (S) lost 26.80 % of weight, representing 96.00% of lignin removed. Mixed sawdust of different woods (M) lost 20.80% weight, representing 80.00% of lignin leached out (Figure 6).



**Figure 6:** Percentage of mass losses after pretreatment with HPAC

### 6.1. Effectiveness and Extent of Delignification Reaction

From the literature, the percentage of lignin in Hardwood and Softwood is 24% and 28%, respectively, while on average, the percentage of lignin in mixed sawdust is 26%. Figure 7 depicts the efficiency, that is, the percentage of effectiveness of the delignification reaction. It was obvious that the delignification reaction is more efficient in delignification in softwood of Ako wood shown to be 96%; this result is possible because softwood is more prone to HPAC attack than Hardwood sawdust of Mahogany tree, which closely followed at 89%, while that of mixed sawdust is the least with a percentage of 80%. This result is enormous because the shield covering the cellulose and hemicellulose from the acid attack has been removed with this high percentage of lignin. Hence, a higher sugar yield during enzymatic hydrolysis is now possible.



**Figure 7:** Percentage effectiveness of delignification using HPAC

## 7. Conclusion

In conclusion, the utilization of Hydrogen Peroxide Acetic Acid at a 1:1 ratio by volume has proven to be highly effective in the removal of lignin during the pretreatment of lignocellulosic biomass, encompassing Ako, Mahogany, and mixed sawdust samples. The substantial lignin removal rates of 96%, 89%, and 80% from Ako tree, Mahogany, and mixed sawdust, respectively, achieved within a mere 30 minutes of reaction time at 80°C, attest to the efficiency of this method.

A pivotal advantage of this approach lies in its ability to prevent the release of inhibitors during subsequent hydrolysis reactions. By effectively mitigating lignin content, a major contributor to inhibitor generation, this pretreatment method obviates the necessity for additional inhibitor separation steps. This simplifies the overall process and significantly bolsters its economic viability.

The implications of these findings are profound, signalling a promising advancement in sustainable biomass utilization. This method presents a compelling solution for enhancing the efficiency and cost-effectiveness of lignocellulosic biomass conversion processes by addressing critical challenges such as lignin removal and inhibitor release.

Moreover, eliminating supplementary separation steps streamlines operations and reduces resource consumption, fostering a more environmentally sustainable approach to biomass conversion.

The results underscore the potential of Hydrogen Peroxide Acetic Acid pretreatment as a pivotal tool in advancing the sustainability and economics of biomass conversion processes. Continued research and development in this area hold promise for further advancements, propelling us toward a greener, more renewable bio-based economy.

Furthermore, the effectiveness and extent of delignification reactions using hydrogen peroxide-acetic acid (HPAC) depend on various factors, including the concentration of hydrogen peroxide and acetic acid, reaction conditions (temperature, pH, reaction time), and the characteristics of the lignocellulosic biomass. Here are the takeaway points of the effectiveness and extent of delignification reactions using HPAC.

**Lignin Removal Efficiency:** HPAC pretreatment effectively removes lignin from lignocellulosic biomass. The hydrogen peroxide acts as an oxidizing agent, breaking down the complex lignin structure, while acetic acid helps solubilize and remove lignin fragments from the biomass matrix. The extent of lignin removal can vary depending on the specific conditions used but is often significant, with removal efficiencies ranging from 80% to 96%.

**Selectivity:** HPAC pretreatment is relatively selective towards lignin removal compared to other components of lignocellulosic biomass, such as cellulose and hemicellulose. While some hemicellulose degradation may occur under harsh reaction conditions, we tried to minimize this by controlling pH and temperature. This selectivity is advantageous as it preserves the integrity of cellulose, which is the primary target for subsequent enzymatic hydrolysis to produce fermentable sugars.

**Reaction Conditions:** The effectiveness of HPAC delignification reactions is highly dependent on the specific reaction conditions employed. Optimal conditions may vary depending on the biomass type and the desired delignification extent. Factors such as temperature, pH, and reaction time must be carefully controlled to balance removing lignin and preserving other biomass components.

**Hydrogen Peroxide and Acetic Acid Concentration:** The concentration of hydrogen peroxide and acetic acid used in the HPAC pretreatment significantly influences the extent of delignification. Higher concentrations of hydrogen peroxide and acetic acid generally lead to more efficient lignin removal but may also increase the risk of side reactions or degradation of other biomass components. Optimization of these concentrations is critical to achieving maximum delignification efficiency while minimizing adverse effects.

**Economic Considerations:** While HPAC pretreatment can be highly effective for delignification, its economic viability depends on factors such as the cost of reagents, energy requirements, and downstream processing costs. Efforts to optimize reaction conditions and minimize reagent consumption are essential to ensure the process remains economically feasible, particularly at industrial scales.

**Environmental Impact:** HPAC pretreatment is generally considered more environmentally friendly than other pretreatment methods, such as acid or alkali treatments, due to its use of relatively mild and environmentally benign reagents. However, managing by-products and waste streams is still necessary to minimize environmental impact.

HPAC pretreatment offers a promising approach for delignifying lignocellulosic biomass, as demonstrated in the research work, with the potential to enhance the efficiency of subsequent biomass conversion processes significantly. Optimization of reaction conditions and careful consideration of economic and environmental factors are essential for maximizing the effectiveness and sustainability of HPAC delignification reactions.

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**Ethics and Consent Statement:** This research adheres to ethical guidelines, obtaining informed consent from all participants. Confidentiality measures were implemented to safeguard participant privacy.

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