

# Enhancement of Enzymatic Hydrolysis and Ethanol Production from Pine Chips by Steam Explosion–KOH Treatment with Agricultural Reuse of Liquid Residues

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Lignocellulosic biomass is a widely available renewable resource, yet its recalcitrant structure limits enzymatic hydrolysis and bioethanol production. This study aimed to develop an efficient pretreatment method for pine wood chips by integrating steam explosion with potassium hydroxide (KOH) treatment and to explore the reuse potential of the resulting liquid waste. Steam explosion disrupted chip structure and exposed cellulose, while subsequent KOH pretreatment selectively removed lignin and hemicellulose, thereby enhancing enzymatic accessibility. Response surface methodology identified optimal conditions of 2.9 min steam explosion, 1.4% KOH, and 21 h treatment time, which yielded a maximum glucose conversion of 30% (based on raw material). The combined process increased ethanol production, with yields approximately sevenfold higher than untreated controls. In addition, liquid waste containing potassium elements promoted the growth of red lettuce, perilla, and green onion, with perilla showing a fivefold increase in fresh weight compared to controls. These findings demonstrate that steam explosion coupled with KOH pretreatment not only improves the efficiency of bioethanol production but also offers a sustainable route for recycling liquid residues as agricultural fertilizer.

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*Keywords:* KOH; Liquid waste; Enzyme hydrolysis; Vegetable crops; Recycling

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## INTRODUCTION

Globally, there is a growing consensus regarding the climate crisis and energy security. Interest in bioenergy as a direct substitute for fossil fuels has increased (Valentine 2011). The United Nations proposed utilizing forest biomass for energy production as a strategy to expand the share and area of sustainably managed forests. Both the International Energy Agency (IEA) and the International Renewable Energy Agency (IRENA) emphasize that undervalued bioenergy will play a critical role in future energy transitions and in establishing a climate-friendly circular carbon economy (Hulio *et al.* 2022). They further highlight that reducing atmospheric CO<sub>2</sub> necessitates a transition from fossil fuels to sustainable forest biomass as a key climate change mitigation measure (Baul *et al.* 2017).

Lignocellulosic biomass composition varies considerably, depending on the source, but it typically consists of approximately 35 to 50% cellulose, 20 to 35% hemicellulose, and 10 to 30% lignin. These ranges encompass a variety of biomass types, including softwoods, hardwoods, and agricultural residues, each of which exhibits distinct structural and chemical characteristics (Das *et al.* 2023). In wood biomass, cellulose forms the primary structural framework of the cell wall, while hemicelluloses are closely associated with cellulose microfibrils through hydrogen

bonding. Lignin is deposited throughout the cell wall, but it is most abundant in the middle lamella and secondary cell wall, where it contributes to mechanical strength, rigidity, and structural integrity (Fry 2010). The industry that produces bioenergy and biochemical materials from biomass is referred to as a biorefinery (Ahorsu *et al.* 2018). Current biorefinery research largely focuses on processes in which cellulose and hemicellulose, the major polysaccharides in biomass, are hydrolyzed into monosaccharides (Takkellapati *et al.* 2018). These monosaccharides are then utilized as substrates for microbial fermentation to produce high-value-added compounds, with ethanol and xylitol produced by yeast serving as representative examples (Carneiro *et al.* 2019). Such processes are commonly referred to as bioconversion processes. However, bioconversion requires complex and diverse treatment steps due to the recalcitrance of lignocellulosic biomass (Kumar *et al.* 2008). The cost contribution of pretreatment varies considerably depending on process configuration and operating conditions. For instance, Jiang *et al.* (2020) reported, in a specific case study, that pretreatment accounted for approximately 22% of the total capital cost. However, from a practical perspective, both capital investment and operating costs, including energy and chemical inputs, are critical factors influencing the economic feasibility of lignocellulosic bioconversion.

The typical composition of conifers is 42 to 45% cellulose, 21 to 24% hemicellulose, 25 to 30% lignin, 1 to 10% extractives, and less than 1% inorganic compounds by weight. Conifer cellulose is a linear polymer of d-glucose units with a high degree of polymerization ( $DP \approx 10,000$ ), has a highly ordered structure, and is partially crystalline (Kandhola *et al.* 2017). Cellulose is intertwined with hemicellulose, which is a short, branched structure composed of partially acetylated glucomannan and galactoglucomannan, with small amounts of xylose and arabinose (Nitsos *et al.* 2017). Conifer lignin is primarily produced from coniferyl alcohol as a precursor. The lignin is a low-methoxy, highly condensed, crystalline, and resistant to degradation type of guaiacyl and *p*-hydroxyphenyl lignin. Therefore, it is important to find an efficient pretreatment method for sugar or ethanol production from conifers with low enzyme accessibility (Wijeyekoon and Vaidya 2021).

The pretreatment of lignocellulosic biomass to produce sugars or ethanol involves physical methods, such as chipping, grinding, and milling of cellulose crystals, as well as physicochemical processes including steam treatment, acid pretreatment, and alkali pretreatment, in addition to biological methods that employ microorganisms to degrade lignin and hemicellulose (Chiaramonti *et al.* 2012). Among physicochemical approaches, acid pretreatment utilizes strong acids such as sulfuric or hydrochloric acid (Panda *et al.* 2010). Although this method achieves high biomass digestibility, it also presents challenges, including acid toxicity and reactor contamination caused by corrosion (Galanopoulos *et al.* 2018). The use of diluted acids, however, mitigates these drawbacks while improving cellulose digestibility. In contrast, the efficiency of alkali pretreatment is highly dependent on lignin content. Its decomposition mechanism primarily involves the saponification of ester bonds between xylan, hemicellulose, and other structural components (Xu *et al.* 2020). Treatment with diluted sodium hydroxide induces biomass swelling, thereby increasing internal surface area, decreasing crystallinity, disrupting structural linkages between lignin and carbohydrates, and fragmenting lignin structures (Jung *et al.* 2020). Despite these advantages, chemical pretreatments also present certain limitations, highlighting the need for the development of more effective alternatives.

Steam explosion is a widely used hydrothermal pretreatment that disrupts this structure through both autohydrolysis and mechanical shearing forces (Jacquet *et al.*, 2015). This process increases the pore volume and specific surface area of the biomass,

thereby enhancing substrate accessibility for cellulase (Grous *et al.* 1986). However, the accumulation of lignin degradation products and pseudo-lignin on the surface during steam explosion can still hinder enzymatic performance (Pielhop *et al.* 2016).

To overcome this, alkaline washing with reagents such as KOH is employed to selectively solubilize lignin and remove inhibitory components. Alkaline treatment induces biomass swelling and the saponification of ester linkages, which significantly improves the enzymatic hydrolysis rate by reducing the non-specific binding of enzymes to lignin (Chandra *et al.* 2007; Lukajtis *et al.* 2018). Furthermore, the removal of these barriers leads to higher fermentable sugar concentrations, which directly correlates with improved fermentation efficiency and bioethanol yields (Sharma *et al.* 2013). Beyond bioconversion, the sustainable management of alkaline waste streams is crucial. Potassium-based alkaline residues have been proposed as potential soil amendments, as potassium is an essential macronutrient for plant physiology, suggesting that these streams can be safely and effectively applied to arable lands to promote crop growth (Jaiswal *et al.* 2016).

Although the combined use of steam explosion and alkaline treatment is not conceptually novel, its practical implementation remains challenged by chemical consumption, process optimization, and management of alkaline liquid residues. In particular, alkaline wash-waters generated during pretreatment are typically regarded as waste streams, despite containing solubilized organic compounds and inorganic elements that may be valorized. From a biorefinery perspective, the reutilization of such streams could improve overall process sustainability if their feasibility and limitations are appropriately assessed. This study focused on the systematic optimization of a steam explosion–KOH pretreatment process using response surface methodology and examine its effects on substrate structure, enzymatic hydrolysis, and fermentation performance. In addition, the potential reuse of alkaline wash-water was explored at a feasibility level through controlled plant growth tests, with the aim of evaluating its applicability as a recyclable byproduct rather than as a fully developed fertilizer. In summary, this study investigated the effect of KOH pretreatment on the enzymatic hydrolysis of pine chips and explored the potential of utilizing the resulting liquid waste to promote plant growth.

## EXPERIMENTAL

### Material

Pine wood was obtained from Punglim, a timber supplier located in Daehwadong, Daejeon, Republic of Korea. The wood was processed into chips of approximately  $5 \times 5 \times 0.5 \text{ cm}^3$  (length  $\times$  width  $\times$  thickness), where the thickness was controlled to ensure uniform heat transfer and effective steam penetration during pretreatment. Chip thickness is known to be a critical parameter influencing pretreatment efficiency. The prepared chips were oven-dried at 60 °C until reaching a constant weight and subsequently stored under ambient conditions. It should be noted that drying was performed for experimental control purposes rather than as a prerequisite for steam explosion.

### Steam Explosion Pretreatment

Wood chips were subjected to steam explosion (Daegu, South Korea) at a pressure of 25 kg·f/cm<sup>2</sup> for 1, 3, or 5 min, after which the slurry was separated by vacuum filtration. Steam explosion pretreatment was carried out using a pilot-scale batch steam explosion reactor with a total volume of 100 L, constructed of high-

pressure stainless steel and equipped with an external steam supply system, temperature and pressure monitoring, and a rapid depressurization discharge valve. Pine wood chips were prepared to a uniform size of  $5 \times 5 \times 0.5$  cm<sup>3</sup> to ensure consistent heat transfer and steam accessibility during pretreatment. For each pretreatment run, the reactor was loaded with 10 kg of biomass (dry weight basis) per batch. This loading corresponded to the effective working capacity of the reactor and enabled uniform pretreatment severity under all operating conditions. The reactor required approximately 1 to 2 min to reach the target temperature and pressure. The steam explosion residence time reported in this study (1 to 5 min) corresponds to the holding time at the setpoint conditions and excludes the heating-up phase. Following steam explosion, the reactor was rapidly depressurized, and the resulting slurry was discharged and collected directly from the reactor outlet. Solid and liquid fractions were separated by vacuum filtration, and the recovered solids were dried at 60 °C to constant weight prior to subsequent processing. The fraction that passed through a 20-mesh sieve but was retained on an 80-mesh sieve was collected and used as the substrate for subsequent alkaline pretreatment. This fraction accounted for approximately 60% of the initial dry biomass, considering mass loss during pretreatment and size classification. The selected fraction was used to ensure uniformity and reproducibility in subsequent alkaline treatment and enzymatic hydrolysis experiments.

Following steam explosion, the recovered solids were oven-dried at 60 °C until reaching a constant weight to minimize residual moisture. The dried samples were subsequently milled, ensuring that size reduction was performed under essentially dry conditions. This step was introduced to enhance the efficiency of subsequent KOH pretreatment by increasing surface area and facilitating reagent penetration. In this study, milling was considered a complementary step within an integrated pretreatment strategy, rather than an independent variable.

### **KOH Treatment**

Pretreatment with potassium hydroxide (0.5%, 1%, and 2%) was performed under static conditions at room temperature for 12 to 24 h. The solid-to-liquid ratio was maintained at 1:20 (w/v) for all experiments (based dry weight). Alkaline pretreatment was performed under static conditions at room temperature to minimize additional thermal and mechanical energy inputs and to allow evaluation of the intrinsic chemical effects of KOH on steam-exploded biomass. Under these mild conditions, extended soaking times (12 to 24 h) were applied to ensure sufficient interaction between the alkaline solution and the pretreated substrate. It should be noted that this treatment protocol was designed for laboratory-scale assessment rather than for direct industrial implementation. Following treatment, the suspension was filtered through Whatman No. 2 filter paper.

### **Enzymatic Hydrolysis**

For enzymatic hydrolysis, 1 g of sample was transferred into a 30-mL test tube and sterilized at 121 °C for 30 min. After cooling on a clean bench, 0.1 M sodium citrate buffer (pH 5.0), 2% sodium azide, 1% TWEEN 80 (polysorbate 80), and Cellic® CTec2 enzyme (a cellulase product from Novozymes, Denmark) at a loading of 130 FPU/glucan were added. Enzymatic hydrolysis was conducted using an enzyme loading of 130 FPU/glucan. Although this dosage exceeds enzyme levels typically considered for industrial application, it was selected to minimize enzyme-related limitations and to ensure that observed differences in hydrolysis performance primarily reflected variations in substrate accessibility and pretreatment effectiveness. Therefore, the applied enzyme loading should be regarded as a benchmarking condition for

comparative evaluation rather than an economically optimized parameter. Hydrolysis was performed at 50 °C with shaking at 210 rpm for 72 h. The reaction mixture was filtered through a 2G3 glass filter, dried in a constant-temperature desiccator at 105 ± 3 °C for 24 h, and weighed to determine enzymatic hydrolysis yield. The extent of enzymatic hydrolysis was initially estimated based on solid weight loss after hydrolysis. This gravimetric approach was used as a supplementary indicator of overall solid conversion. However, it should be noted that weight loss measurements do not directly quantify soluble sugar production and may be influenced by factors such as lignin solubilization and fine particle loss. Accordingly, the interpretation of hydrolysis efficiency in this study was primarily based on glucose concentrations determined by HPLC rather than on gravimetric weight loss alone. While weight loss data provide some insight into solid degradation behavior, more precise quantitative evaluation of enzymatic performance and downstream fermentation efficiency was conducted using sugar release and ethanol production as the principal metrics.

### Optimization of Steam Explosion-KOH Treatment

Optimization of the combined steam explosion–KOH pretreatment was conducted using response surface methodology (RSM) with Design-Expert software (version V1 3.0.5.0, Stat-Ease, Inc., Minneapolis, MN, USA) based on a Box–Behnken design. The independent variables included steam explosion time (A), KOH concentration (B), and KOH treatment duration (C), while enzymatic hydrolysis efficiency, expressed as glucose yield, was used as the response variable. A total of 17 runs were carried out. Statistical significance was assessed by analysis of variance (ANOVA), with the model fitted to distinguish between  $R^2$  and adjusted  $R^2$  values. The p-values were used to evaluate statistical significance. After pretreatment, the KOH solution was recovered and subsequently applied in crop growth tests.

### Ethanol Fermentation

*Saccharomyces cerevisiae* was inoculated into the enzymatic hydrolysate supplemented with 1% (w/v) yeast extract, 2% (w/v) peptone, and 4% (w/v) dextrose. The inoculum size was standardized to 5 g wet weight of yeast per liter of hydrolysate. Fermentation was carried out at 30 °C and 150 rpm for 72 h. The resulting culture was centrifuged at 12,000 rpm for 1 min, and the supernatant was collected for ethanol quantification.

### Analysis Methods

The lignin content of wood samples was determined following the TAPPI T222 om-11 standard (2011) protocol, while sugar content was quantified according to the NREL standard method (Sluiter *et al.* 2008).

Wide-angle X-ray diffraction (XRD) patterns were obtained using an X-ray diffractometer (Ultima IV, Rigaku, Japan). Measurements were obtained with Cu-K $\alpha$  radiation ( $\lambda = 0.1542$  nm) at 40 kV and 25 mA. Diffraction intensities were collected over a  $2\theta$  range of 10 to 90° with a step size of 0.02°. The crystallinity index (CrI) was calculated using the following Eq. 1,

$$\text{CrI} = (I_{002} - I_{\text{am}}) / I_{002} \times 100 \quad (1)$$

where  $I_{002}$  is the intensity of the (002) plane at approximately  $2\theta = 22.5^\circ$ , and  $I_{\text{am}}$  is the intensity of the baseline at approximately  $2\theta = 18.4^\circ$ .

Samples were oven-dried at 30 °C for 24 h and stored under ambient conditions. After degassing for 5 days at room temperature, the Brunauer–Emmett–Teller (BET) surface area was determined at a relative pressure of approximately 0.994

using a Micromeritics ASAP 2020 analyzer (Micromeritics, Norcross, GA, USA).

Glucose concentration in the enzymatic hydrolysates was quantitatively determined using high-performance liquid chromatography (HPLC). After enzymatic hydrolysis, reaction mixtures were centrifuged at 12,000 rpm for 5 min to remove residual solids. The supernatants were subsequently filtered through 0.22  $\mu\text{m}$  syringe filters (PTFE) prior to analysis.

HPLC analysis was performed using an Agilent 1260 Infinity system (Agilent Technologies, Santa Clara, CA, USA) equipped with a refractive index detector (RID). Separation was achieved using an Aminex HPX-87H column (300  $\times$  7.8 mm, Bio-Rad Laboratories, Hercules, CA, USA) maintained at 60  $^{\circ}\text{C}$ . The mobile phase consisted of 5 mM  $\text{H}_2\text{SO}_4$ , delivered at a flow rate of 0.6 mL/min. The injection volume was 20  $\mu\text{L}$ .

Glucose was identified and quantified by comparing retention times and peak areas with those of authentic glucose standards prepared at known concentrations. Calibration curves were generated using at least five standard concentrations, and linearity was confirmed with coefficients of determination ( $R^2$ ) greater than 0.999. All measurements were conducted in triplicate, and glucose concentrations were reported as mean values  $\pm$  standard deviation.

Enzymatic hydrolysis efficiency was primarily evaluated based on glucose concentration determined by HPLC. Gravimetric weight loss measurements were used only as supplementary information to describe overall solid degradation behavior.

Ethanol concentration was analyzed using high-performance liquid chromatography (HPLC, 1260 Infinity, Agilent Technologies, Santa Clara, CA, USA) equipped with an Aminex HPX-87H column (Bio-Rad, 60  $^{\circ}\text{C}$ ). The mobile phase was 5 mM  $\text{H}_2\text{SO}_4$ , applied at a flow rate of 0.6 mL/min.

### Liquid Waste Used for Culturing Crops

Liquid waste was obtained from pine chips pretreated with 1% KOH solution after 3 min of steam explosion. Both undiluted and 50% diluted solutions were prepared and applied in pot tests. Three crops were tested: red lettuce, perilla, and green onion. Growth medium was prepared by mixing commercial potting soil and vermiculite at a 3:1 (v/v) ratio. One week after sowing, either undiluted liquid waste, diluted liquid waste, or water (control) was applied to pots (60  $\times$  6  $\times$  50  $\text{cm}^3$ ; 5 mL/pot). After 60 days of cultivation, fresh weight, plant height, leaf length, and root length were recorded.

### Statistical Calculation

All statistical analyses were performed using SAS software (SAS Institute Inc., Chicago, IL, USA). Pairwise comparisons between treated and control groups were conducted using Student's t-test. Data are presented as mean values derived from raw measurements.

## RESULTS AND DISCUSSION

### Effect of Steam Explosion-KOH Treatment on Composition

The dry weight of pine chips was reduced approximately 24% following steam explosion, which is consistent with earlier studies reporting a 4 to 27% loss in wood chip mass after this treatment (Shimizu *et al.* 1998). This loss is mainly attributed to volatilization and solubilization of hemicellulose-derived sugars, organic acids, and other low-molecular-weight compounds formed during autohydrolysis and rapid depressurization. Steam explosion also caused a marked decrease in hemicellulose, resulting in a relative increase in lignin proportion. This observation agrees with Chua

and Wayman (1979), who supported Klemoda's hypothesis that under harsh pretreatment conditions, reactive degradation products of hemicellulose, such as furfural and its precursors, can undergo condensation reactions with lignin during autohydrolysis. Rather than representing a loss of material from the system, this apparent mass reduction reflects a redistribution of biomass components among different phases. During rapid heating and depressurization, a fraction of the biomass is released as volatile compounds and gases, while another portion is solubilized into the liquid phase as hemicellulose-derived sugars, organic acids, and lignin-derived fragments. Only the remaining fraction is recovered as solid material. Under the conditions applied in this study, steam explosion primarily induced autohydrolysis and physical disruption of the biomass matrix, rather than thermochemical conversion processes such as carbonization or torrefaction. Similar interpretations of mass loss mechanisms during steam explosion have been reported in the literature, where volatilization and solubilization are identified as the dominant contributors to reduced solid recovery (Manouchehrinejad and Mani 2018; Pažitný 2019; Pažitný *et al.* 2020). Two major types of reactions occur during steam explosion: initially, rapid depolymerization of native lignin and hemicellulose through acid hydrolysis, followed by condensation and repolymerization as heating continues, leading to an increase in acid-insoluble (Klason lignin) residues. Previous studies have similarly reported lignin degradation and structural modification during the autohydrolysis of lignocellulosic biomass (Moniruzzaman 1996). It should be noted that the sum of quantified structural carbohydrates and lignin does not equal 100%. The unaccounted fraction (approximately 25% after alkaline treatment) includes extractives, ash, water-soluble lignin fragments, degradation products derived from hemicellulose and lignin, and other minor components not captured by the standard compositional analysis. Similar mass closures have been reported in studies involving alkaline pretreatment, where partial solubilization and degradation of biomass components occur.

**Table 1.** Composition (% Based Dry Weight) in Raw and Treated Pine Wood Chip

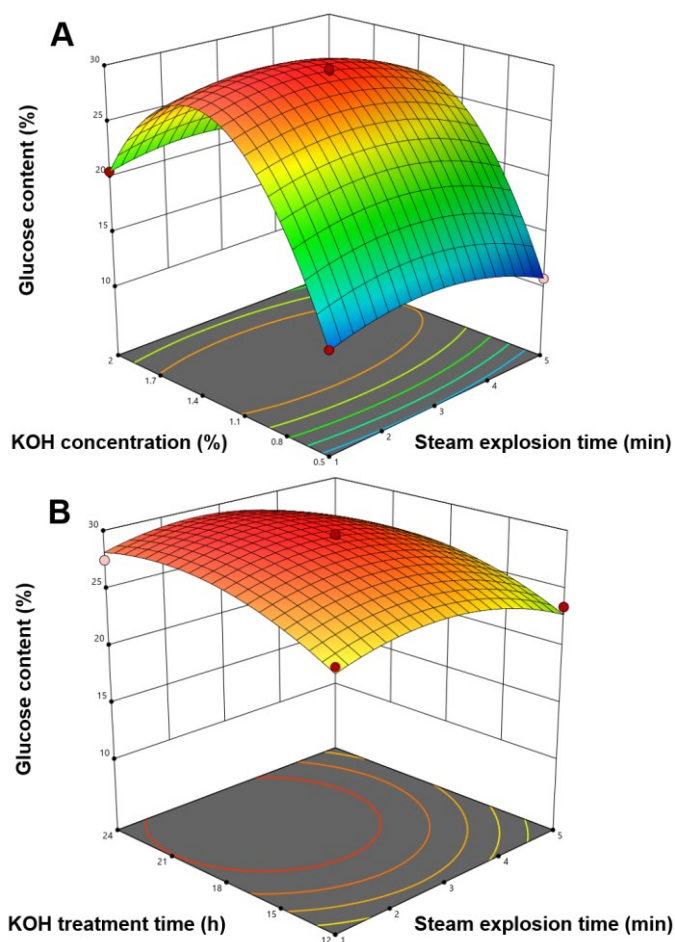
Treatment	Solid Recovery	Glucan	Xylan	Arabinosyl Residues	Lignin
Raw	-	44.5 ± 0.1	14.2 ± 0.0	4.1 ± 0.0	35.7 ± 0.1
Steam explosion (3 min)	75.9 ± 0.0 (based raw)	44.2 ± 0.1	6.7 ± 0.0	1.8 ± 0.0	58.9 ± 0.7
KOH after steam explosion (1.4%, 21 h)	61.4 ± 0.0 (based raw)	47.5 ± 0.2	0.8 ± 0.1	0.3 ± 0.1	26.4 ± 0.1

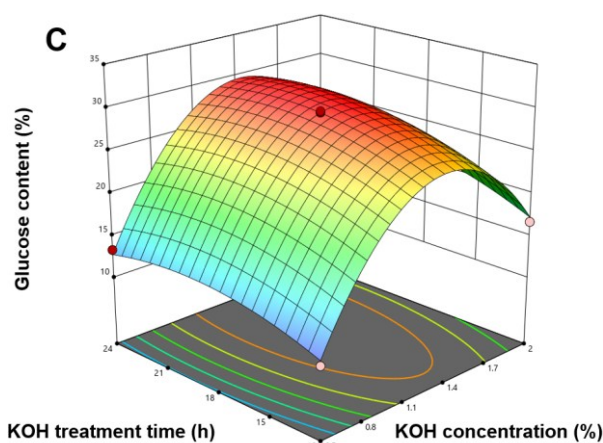
A reduction in lignin content was detected following KOH treatment (Table 1). This reaction decreases the molecular weight of lignin, enhances its solubility, and facilitates its release from the cell wall (Song *et al.* 2019). In addition, during alkaline treatment, methoxy groups ( $-OCH_3$ ) in lignin are partially removed or transformed into phenolic hydroxyl groups ( $-OH$ ). Such modifications increase the hydrophilicity and ionization of lignin, promoting its migration into the aqueous phase (Awoyale and Lokhat 2021). The prolonged alkaline soaking employed in this study should be regarded as a controlled experimental approach rather than an industrially optimized pretreatment strategy. Static treatment at ambient temperature with extended residence time is not considered practical at large scale. Future process development should therefore focus on reducing treatment duration through the application of agitation,

moderate heating, or continuous-flow reactor designs, while maintaining comparable delignification efficiency. The maximum glucose yield reported in this study (30% on a raw-material basis) represents glucose production normalized to the initial dry mass of raw biomass. This raw-basis metric is conservative because it inherently accounts for pretreatment-related solid losses and therefore should not be interpreted as the cellulose-to-glucose conversion efficiency. To enable clearer comparison with literature and to better reflect enzymatic performance, glucose release was additionally expressed as g glucose per g initial glucan and percent of theoretical glucose yield based on initial glucan content. The glucose production yield was 0.68 g of glucose per 1 g of initial glucan and 68% of the theoretical glucose yield based on the initial glucan content. These additional metrics provide a more direct indication of carbohydrate conversion, while the raw-basis yield reflects the overall process outcome including pretreatment mass balance.

### Model Optimization of Steam Explosion and KOH Treatment on Pine Wood Chips

Three experimental factors, steam explosion duration (A), KOH concentration (B), and KOH treatment duration (C), were identified as critical variables influencing the enzymatic hydrolysis efficiency of pine chips. A Box–Behnken design was employed, with these three factors set as independent variables and glucose yield after enzymatic hydrolysis designated as the response. Following hydrolysis under the specified conditions, response surface methodology (RSM) was applied to determine the optimal pretreatment parameters for maximizing glucose release (Fig. 1).





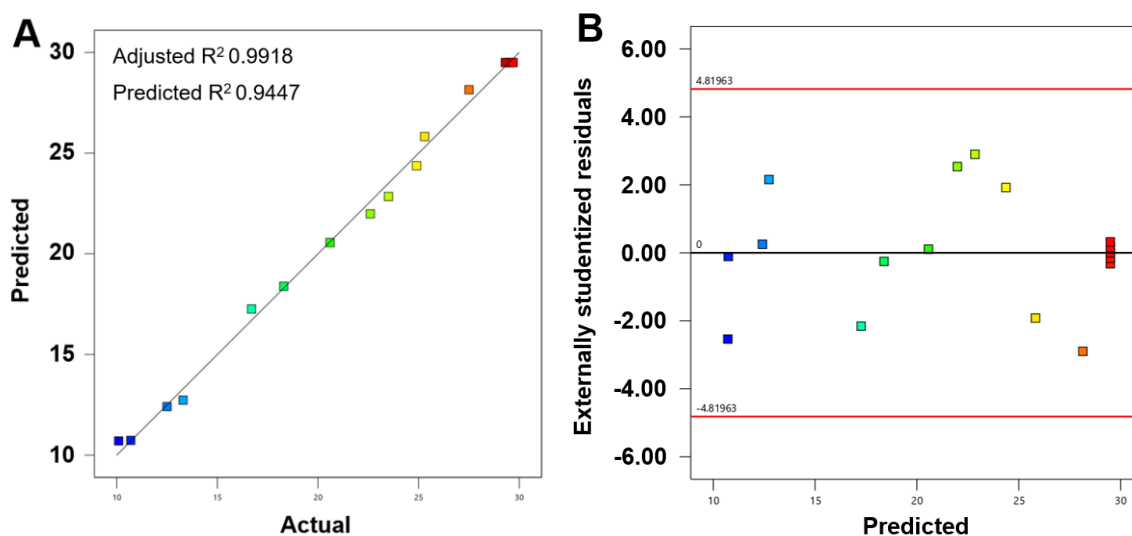
**Fig. 1.** A response surface and contour plot constructed based on the Y model, illustrating interactions between variables, with the generated glucose content as the response variable: A: steam explosion time - KOH concentration; B: steam explosion time - KOH treatment time; C: KOH treatment time - KOH treatment time

Analysis of variance (ANOVA) indicated that a cubic regression model best described the relationship between pretreatment variables and glucose yield. The resulting predictive equation for glucose yield is presented below,

$$\begin{aligned} \text{Glucose content (\% based raw material)} = & + 29.50 - 0.9625A + 3.95B \\ & + 1.69C - 0.125AB - 0.2AC + 0.675BC - 2.18A^2 - 11.8B^2 - 2.03C^2 \end{aligned} \quad (1)$$

where A represents steam explosion time, B represents KOH concentration, and C represents KOH treatment time.

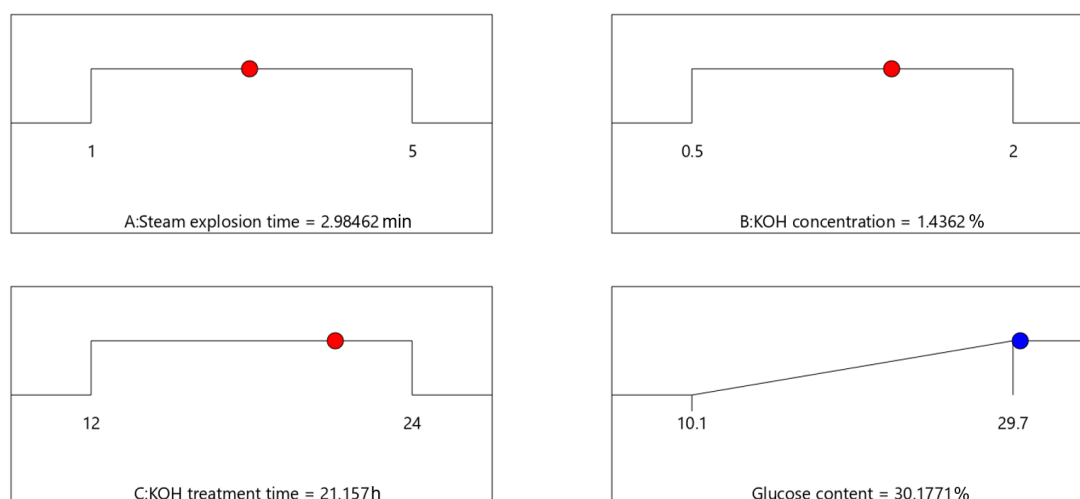
To evaluate the adequacy of the reduced models, actual *versus* predicted plots and residual distributions were examined (Fig. 2). In these analyses, both simplified models produced predicted responses that closely matched the experimental values. As shown in Fig. 2A, the predicted glucose yields were in close agreement with the experimental values, as supported by a high coefficient of determination ( $R^2$ ). This quantitative parameter confirms the adequacy of the fitted model in describing the observed variability in enzymatic hydrolysis performance.



**Fig. 2.** Explain variability in enzymatic hydrolysis results of pine chips using steam explosion and KOH treatment conditions: A: Actual  $\times$  predicted value plot; B: residue distribution

The lack-of-fit value was found to be insignificant. To further verify the adequacy of the reduced models, plots of actual *versus* predicted values along with the residual plot shown in Fig. 2B were analyzed. These plots demonstrated that the predicted responses from both reduced models were in close agreement with the experimental results. The maximum glucose yield (30% based on raw material) was obtained under central point conditions: steam explosion time of 2.9 min, KOH concentration of 1.4%, and KOH treatment duration of 21.1 h (Fig. 3).

Based on this statistically validated model, Fig. 3 presents a visual representation of the optimized pretreatment conditions predicted to maximize glucose yield. The optimal preprocessing conditions in Fig. 3 were suggested by the RSM model, and the results for the reliability of this model are shown in Table 2. As shown in Table 2, the adjusted  $R^2$  and predicted  $R^2$  values of the model (0.9918 and 0.9447, respectively) exhibited strong agreement with the experimental data. An  $R^2$  value of 0.9447 indicates that 94.47% of the variation in glucose yield was explained by the reduced model. Analysis of variance (ANOVA) further revealed that KOH concentration had the most significant influence on glucose yield ( $p < 0.0001$ ), which is consistent with the trend illustrated in the contour plot (Figs. 2 and 3).



**Fig. 3.** Optimized solution for pine chip processing conditions to maximize glucose content

**Table 2.** ANOVA of Optimized Model Obtained with Box-Behnken Design

Source	F-value	<i>p</i> -value
Model	217.32	< 0.0001
A	17.90	0.0039
B	301.55	< 0.0001
C	55.04	0.0001
AB	0.1510	
AC	0.3865	0.5538
BC	4.40	0.0741
A <sup>2</sup>	48.12	0.0002
B <sup>2</sup>	1416.36	< 0.0001
C <sup>2</sup>	41.74	0.0003
R <sup>2</sup>	0.9964	
Adjusted R <sup>2</sup>	0.9918	
Predicted R <sup>2</sup>	0.9447	

### Effect of Steam Explosion-KOH Treatment on Glucose Content

The highest glucose yield was obtained from pine chips subjected to KOH treatment following steam explosion, compared with untreated pine chips. Under these conditions, glucose release was approximately 1.7-fold greater than that of the raw material. This improvement can be attributed to the reduced lignin content, because lignin removal alleviates steric hindrance and thereby enhances enzymatic accessibility. These findings confirm that lignin content is a critical factor influencing hydrolysis efficiency, as lignin functions as a physical barrier that restricts enzyme interaction with cellulose (Kim and Holtzapfle 2006). Notably, the addition of polysorbate 80 also enhanced glucose production.

As shown in Fig. 4, the addition of polysorbate 80 resulted in a statistically significant increase in glucose release compared to the corresponding treatments without surfactant ( $p < 0.001$ ). However, the absolute increase in glucose yield was relatively small. This indicates that, although polysorbate 80 exerted a measurable effect under the conditions tested, its contribution to enhanced enzymatic hydrolysis was limited in practical terms. Therefore, in the present study, polysorbate 80 is considered to have a supplementary rather than a dominant role in influencing enzyme accessibility, particularly when compared to the effects of pretreatment severity and lignin removal.

Previous studies have demonstrated that polysorbate 80 lowers surface tension during pretreatment, promoting proton penetration into lignocellulosic structures and improving access to hemicellulose and lignin (Wang *et al.* 2020). Similarly, Qing *et al.* (2010) reported that surfactant supplementation can facilitate lignin removal by forming emulsions that capture degraded lignin fragments, thereby reducing their re-deposition onto biomass surfaces. The maximum glucose yield reported in this study (30% on a raw-material basis) represents glucose production normalized to the initial dry mass of raw biomass. This raw-basis metric is conservative because it inherently accounts for pretreatment-related solid losses and therefore should not be interpreted as the cellulose-to-glucose conversion efficiency.

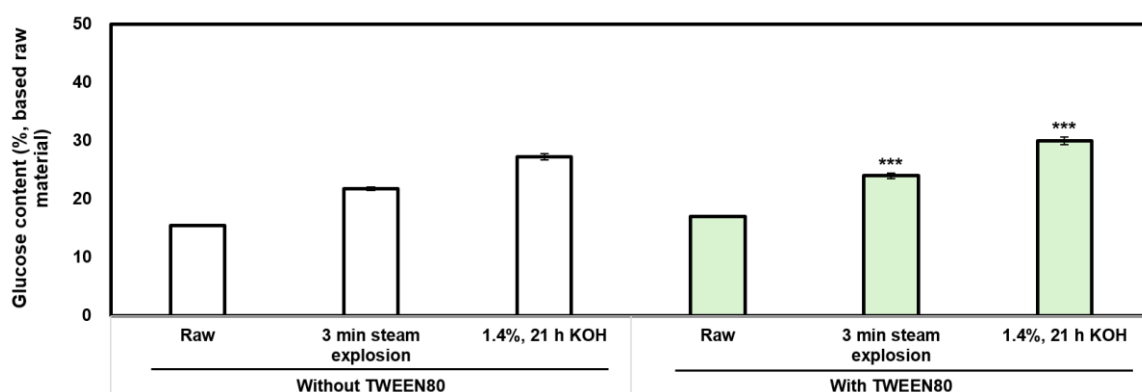
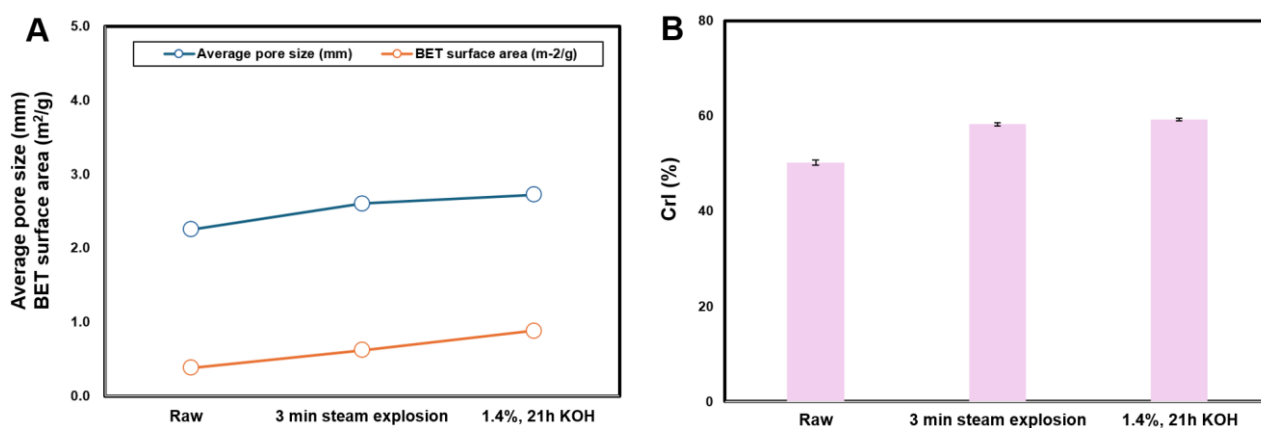


Fig. 4. Glucose content of raw and treated pine chip with and without 1% polysorbate 80; \*\*\*:  $p < 0.001$  compared to without polysorbate 80; 1.4%, 21 h KOH: treated pine chip after 3 min steam explosion

### Change of Microstructure Characteristics and Crystallinity

The influence of steam explosion–KOH pretreatment on the porosity and specific surface area of wood chips was examined. As shown in Fig. 5A, mass loss, average pore size, and BET surface area changed concurrently with increasing pretreatment severity.



**Fig. 5.** Effect of steam explosion-KOH treatment on the specific surface area (A) and CrI (B) of pine chips. 1.4%, 21 h KOH: treated pine chip after 3 min steam explosion

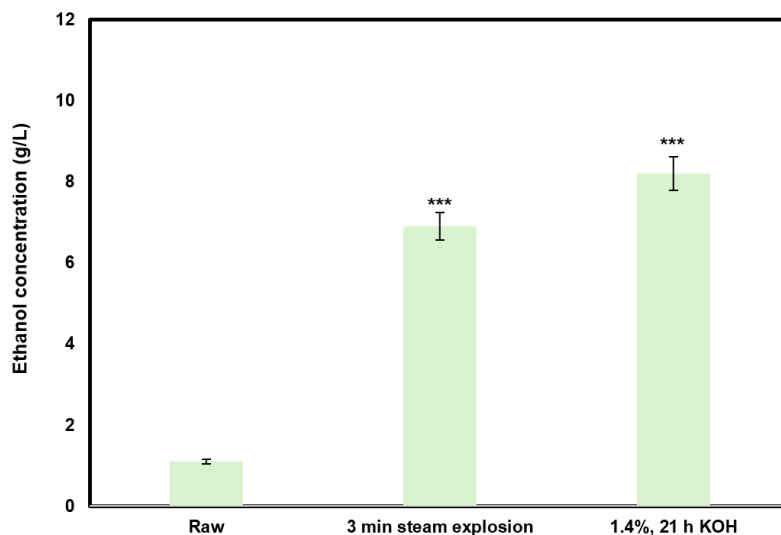
Although these parameters display similar trends, the data do not establish a strong quantitative correlation. Instead, the results suggest that pretreatment-induced mass loss is accompanied by microstructural changes, including increased pore development and surface area. Consistent with previous reports, the removal of hemicellulose and lignin from the cell wall during pretreatment generated voids, enlarged pore size, and opened the chip structure, thereby increasing specific surface area (Lee *et al.* 2017). These results indicate that the elimination of structural components—particularly hemicellulose—significantly enhanced the porosity and surface area of pretreated chips. Increased porosity facilitates deeper penetration of chemical reagents, while a larger surface area promotes interfacial interactions between the biomass and reagents, ultimately improving delignification efficiency.

The crystallinity index (CrI) of untreated pine chips was 50.22%, reflecting the abundance of amorphous cellulose regions and the presence of amorphous constituents such as lignin and hemicellulose (Debiagi *et al.* 2020). The CrI increased after steam explosion-KOH pretreatment. However, this increase should be interpreted primarily as a consequence of the removal of amorphous components, such as lignin and hemicellulose, rather than as a direct change in the crystalline structure of cellulose (Tian *et al.* 2017). Therefore, in the present study, CrI is considered a secondary indicator that reflects compositional shifts induced by pretreatment, rather than an independent structural parameter governing enzymatic hydrolysis. The removal of these amorphous fractions permits cellulose chains to align more closely, thereby promoting coalescence or co-crystallization of cellulose microfibrils with primary fibers.

### Effect of Steam Explosion-KOH Treatment on Ethanol Yield

The amount of fermentable sugars, primarily glucose, increased after pretreatment and enzymatic hydrolysis, and these sugars were subsequently utilized by yeast to produce bioethanol. Pine chips subjected to steam explosion followed by KOH treatment yielded higher ethanol concentrations (g/L) than those treated with steam explosion alone, and approximately seven times more than raw pine chips (Fig. 6), because of the greater glucose release. The combined steam explosion-alkali process improved enzymatic digestibility, which in turn enhanced ethanol production during yeast fermentation. In summary, integrating steam explosion with KOH pretreatment effectively increased both glucose yield and bioethanol output, demonstrating its potential as a viable strategy for the bioconversion of lignocellulosic biomass. The authors acknowledge that ethanol titer is a key parameter for downstream separation and process feasibility. The fermentation experiments in this work were intended to

verify the relative effect of pretreatment on fermentable sugar availability and ethanol formation under controlled conditions, rather than to achieve distillation-ready titers. Achieving industrially relevant ethanol concentrations would require further optimization, including higher-solids hydrolysis/fermentation, reduced residence time, and enzyme loading optimization, as well as process integration strategies.



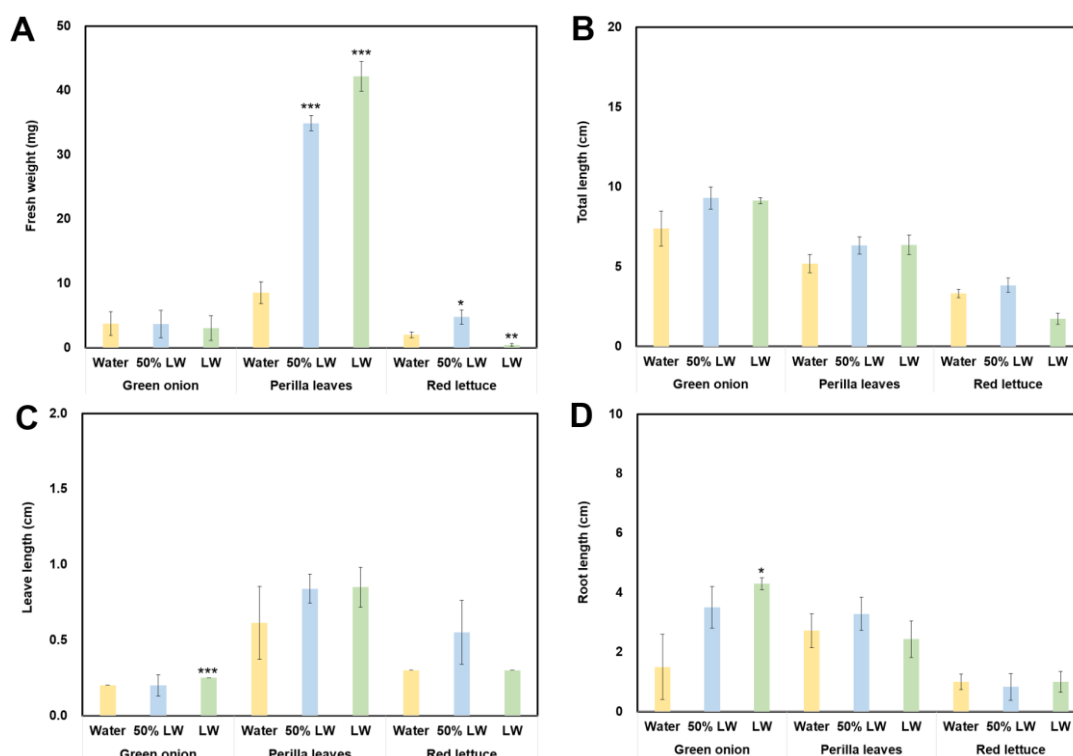
**Fig. 6.** Ethanol production after enzymatic hydrolysis of raw and treated pine chip. \*\*\*:  $p < 0.001$  compared raw; 1.4%, 21h KOH: pine chip after 3 min steam explosion

### Effect of Liquid Waste From KOH Pretreatment on Crop Growth

Based on the above findings, liquid waste obtained after pine chip pretreatment was collected and evaluated for potential reuse as fertilizer. This study examined the feasibility of applying the liquid waste to agricultural production. Liquid waste derived from 1% KOH-treated pine chips was diluted to 50% (v/v) with water and applied to three crop species—green onion, red lettuce, and perilla. Crops receiving only water served as controls. The results demonstrated that all crops treated with liquid waste exhibited significant increases in shoot and root biomass without any adverse effects (Fig. 7).

Although statistical significance was not observed at every interval, the highest average values of total length, leaf length, and root length across all crops were obtained with liquid waste treatment (either 50% diluted or undiluted). In particular, perilla treated with liquid waste showed a fivefold increase in fresh weight compared to the control. These results suggest that liquid waste generated during KOH pretreatment holds considerable promise as a recycled fertilizer for promoting crop growth. Furthermore, bioethanol production technology based on KOH pretreatment could be developed with the additional benefit of producing recyclable liquid byproducts. Future studies should clarify the role of other components, such as lignin- and hemicellulose-derived degradation products, in plant growth and assess the effects of liquid waste on soil properties.

The plant growth experiments conducted in this study were designed as a preliminary feasibility assessment rather than a comprehensive agronomic evaluation. While the alkaline wash-water promoted biomass accumulation in all tested crops without observable adverse effects, these results should be interpreted as proof-of-concept evidence for potential reuse of pretreatment effluents.



**Fig. 7.** Crop growth under the application of liquid waste from KOH pretreatment. Three different crops (green onion, red lettuce, and perilla leaves) were respectively cultured in pots with addition of water, 50% LW, and LW. A–D: Fresh weight, total length, leaf length, root length. \*, \*\* and \*\*\*: significant difference analyzed by t-test at  $p < 0.05$ ,  $0.01$ , and  $0.001$  between water (control) and LW treatments. Bars indicated means  $\pm$  SD ( $n = 3$ ).

The observed growth enhancement is likely attributable to the presence of potassium ions and water-soluble organic compounds released during alkaline treatment; however, the specific contribution of individual components was not quantified in this study. Moreover, long-term effects on soil properties, nutrient balance, and environmental safety were not assessed. Therefore, the present findings demonstrate the practical potential of alkaline wash-water reuse within a circular biorefinery framework, while highlighting the need for further investigation prior to field-scale application.

KOH recovery and reuse will be critical for improving the economic and environmental sustainability of alkaline-based pretreatment. In future work, a closed-loop reuse strategy will be evaluated, including filtration of the spent liquor after alkaline extraction, adjustment of effective alkalinity, and reuse over multiple pretreatment cycles. Practical approaches will include direct liquor reuse with make-up KOH to restore alkalinity, removal of dissolved organics through pH adjustment and separation of lignin-rich fractions, and concentration-based strategies to manage liquor strength and the accumulation of solubilized compounds.

In conclusion, pine wood chips pretreated with 1% KOH after steam explosion were identified as the most effective condition for enhancing enzymatic hydrolysis efficiency. To improve the economic viability of KOH-based pretreatment, strategies for KOH recovery and reuse should be considered. Future studies need to assess both the effectiveness and scalability of such recovery methods within an integrated pretreatment framework. Furthermore, detailed analysis of the filtrate obtained after KOH treatment is necessary to characterize the solubilized fractions of lignin and other organic compounds. The development and application of appropriate separation technologies for KOH recycling will be critical to advancing the overall economic and

environmental sustainability of the process. Overall, the significance of this study lies not in introducing a new pretreatment concept, but in integrating pretreatment optimization, hydrolysis and fermentation performance, and byproduct reutilization within a single experimental framework. This integrated approach provides a practical perspective on improving the sustainability of lignocellulosic bioconversion processes.

## CONCLUSIONS

1. A two-step approach, consisting of steam explosion followed by KOH post-treatment, was applied to enhance the enzymatic hydrolysis of pine wood chips.
2. The steam explosion process disrupted the chip structure, exposing cellulose surfaces as pretreatment pressure and duration increased, thereby facilitating cellulase enzyme binding.
3. A maximum glucose yield of 30% (based on raw material) was obtained under conditions of 1% KOH treatment for 21 h at room temperature, following steam explosion.
4. The integration of steam explosion with KOH post-treatment proved to be an effective strategy for fractionating and converting the major constituents of pine wood chips into a range of bio-based products within a biorefinery framework.

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## REFERENCES CITED

- Ahorsu, R., Medina, F., and Constantí, M. (2018). "Significance and challenges of biomass as a suitable feedstock for bioenergy and biochemical production: A review," *Energies* 11(12), article 3366. <https://doi.org/10.3390/en11123366>
- Awoyale, A. A., and Lokhat, D. (2021). "Experimental determination of the effects of pretreatment on selected Nigerian lignocellulosic biomass in bioethanol production," *Scientific Reports* 11(1), article 557. <https://doi.org/10.1038/s41598-020-78105-8>
- Baul, T. K., Alam, A., Ikonen, A., Strandman, H., Asikainen, A., Peltola, H., and Kilpeläinen, A. (2017). "Climate change mitigation potential in boreal forests: Impacts of management, harvest intensity and use of forest biomass to substitute fossil resources," *Forests* 8(11), article 455. <https://doi.org/10.3390/f8110455>
- Carneiro, C. V. G., Silva, F. C. D. P. E., and Almeida, J. R. (2019). "Xylitol production: Identification and comparison of new producing yeasts," *Microorganisms* 7(11), article 484. <https://doi.org/10.3390/microorganisms7110484>
- Chandra, R. P., Bura, R., Mabee, W. E., Berlin, A., Pan, X., and Saddler, J. N. (2007). "Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulose?," *Advances in Biochemical Engineering/Biotechnology* 108, 67-93. [https://doi.org/10.1007/10\\_2007\\_064](https://doi.org/10.1007/10_2007_064)

- Chiaromonti, D., Prussi, M., Ferrero, S., Oriani, L., Ottonello, P., Torre, P., and Cherchi, F. (2012). "Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method," *Biomass and Bioenergy* 46, 25-35. <https://doi.org/10.1016/j.biombioe.2012.04.020>
- Chua, M. G., and Wayman, M. (1979). "Characterization of autohydrolysis aspen (*P. tremuloides*) lignins. Part 1. Composition and molecular weight distribution of extracted autohydrolysis lignin," *Canadian Journal of Chemistry* 57(10), 1141-1149. <https://doi.org/10.1139/v79-187>
- Das, N., Jena, P. K., Padhi, D., Kumar Mohanty, M., and Sahoo, G. (2023). "A comprehensive review of characterization, pretreatment and its applications on different lignocellulosic biomass for bioethanol production," *Biomass Conversion and Biorefinery* 13(2), 1503-1527. <https://doi.org/10.1007/s13399-021-01294-3>
- Debiagi, F., Madeira, T. B., Nixdorf, S. L., and Mali, S. (2020). "Pretreatment efficiency using autoclave high-pressure steam and ultrasonication in sugar production from liquid hydrolysates and access to the residual solid fractions of wheat bran and oat hulls," *Applied Biochemistry and Biotechnology* 190(1), 166-181. <https://doi.org/10.1007/s12010-019-03092-0>
- Fry, S. C. (2010). "Cell wall polysaccharide composition and covalent crosslinking," *Annual Plant Reviews: Plant Polysaccharides, Biosynthesis and Bioengineering* 41, 1-42. <https://doi.org/10.1002/9781444391015.ch1>
- Galanopoulos, C., Yan, J., Li, H., and Liu, L. (2018). "Impacts of acidic gas components on combustion of contaminated biomass fuels," *Biomass and Bioenergy* 111, 263-277. <https://doi.org/10.1016/j.biombioe.2017.04.003>
- Grous, W. R., Converse, A. O., and Grethlein, H. E. (1986). "Effect of steam explosion pretreatment on pore size and enzymatic hydrolysis of poplar," *Enzyme and Microbial technology* 8(5), 274-280. [https://doi.org/10.1016/0141-0229\(86\)90021-9](https://doi.org/10.1016/0141-0229(86)90021-9)
- Hulio, Z. H., Jiang, W., and Chandio, G. S. (2022). "Power policies, challenges, and recommendations of renewable resource assessment in Pakistan," *Energy Exploration & Exploitation* 40(3), 947-976. <https://doi.org/10.1177/01445987211064678>
- Jacquet, N., Maniet, G., Vanderghem, C., Delvigne, F., and Richel, A. (2015). "Application of steam explosion as pretreatment on lignocellulosic material: A review," *Industrial & Engineering Chemistry Research* 54(10), 2593-2598. <https://doi.org/10.1021/ie503151g>
- Jaiswal, D. K., Verma, J. P., Prakash, S., Meena, V. S., and Meena, R. S. (2016). "Potassium as an important plant nutrient in sustainable agriculture: A state of the art," in: *Potassium Solubilizing Microorganisms for Sustainable Agriculture*, V. S. Meena, et al. (eds.), pp. 21-29. [https://doi.org/10.1007/978-81-322-2776-2\\_2](https://doi.org/10.1007/978-81-322-2776-2_2)
- Jiang, X., de Assis, C. A., Kollman, M., Sun, R., Jameel, H., Chang, H. M., and Gonzalez, R. (2020). "Lignin fractionation from laboratory to commercialization: Chemistry, scalability and techno-economic analysis," *Green Chemistry* 22(21), 7448-7459. <https://doi.org/10.1039/D0GC02960B>
- Jung, W., Savithri, D., Sharma-Shivappa, R., and Kolar, P. (2020). "Effect of sodium hydroxide pretreatment on lignin monomeric components of *Miscanthus × giganteus* and enzymatic hydrolysis," *Waste and Biomass Valorization* 11(11), 5891-5900. <https://doi.org/10.1007/s12649-019-00859-8>
- Kandhola, G., Djiroleu, A., Carrier, D. J., and Kim, J. W. (2017). "Pretreatments for enhanced enzymatic hydrolysis of pinewood: A review," *BioEnergy research* 10(4), 1138-1154. <https://doi.org/10.1007/s12155-017-9862-3>
- Kim, S., and Holtzapple, M. T. (2006). "Effect of structural features on enzyme

- digestibility of corn stover,” *Bioresource Technology* 97(4), 583-591.  
<https://doi.org/10.1016/j.biortech.2005.03.040>
- Kumar, R., Singh, S., and Singh, O. V. (2008). “Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives,” *Journal of Industrial Microbiology and Biotechnology* 35(5), 377-391. <https://doi.org/10.1007/s10295-008-0327-8>
- Lee, H. R., Kazlauskas, R. J., and Park, T. H. (2017). “Mild pretreatment of yellow poplar biomass using sequential dilute acid and enzymatically-generated peracetic acid to enhance cellulase accessibility,” *Biotechnology and Bioprocess Engineering* 22(4), 405-412. <https://doi.org/10.1007/s12257-017-0139-7>
- Łukajtis, R., Rybarczyk, P., Kucharska, K., Konopacka-Łyskawa, D., Słupek, E., Wychodnik, K., and Kamiński, M. (2018). “Optimization of saccharification conditions of lignocellulosic biomass under alkaline pre-treatment and enzymatic hydrolysis,” *Energies* 11(4), article 886. <https://doi.org/10.3390/en11040886>
- Manouchehrinejad, M., and Mani, S. (2018). “Torrefaction after pelletization (TAP): Analysis of torrefied pellet quality and co-products,” *Biomass and Bioenergy* 118, 93-104. <https://doi.org/10.1016/j.biombioe.2018.08.015>
- Moniruzzaman, M. (1996). “Effect of steam explosion on the physicochemical properties and enzymatic saccharification of rice straw,” *Applied Biochemistry and Biotechnology* 59(3), 283-297. <https://doi.org/10.1007/BF02783570>
- Nitsos, C., Rova, U., and Christakopoulos, P. (2017). “Organosolv fractionation of softwood biomass for biofuel and biorefinery applications,” *Energies* 11(1), article 50. <https://doi.org/10.3390/en11010050>
- Panda, A. K., Mishra, B. G., Mishra, D. K., and Singh, R. K. (2010). “Effect of sulphuric acid treatment on the physico-chemical characteristics of kaolin clay,” *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 363(1-3), 98-104. <https://doi.org/10.1016/j.colsurfa.2010.04.022>
- Pažitný, A. (2019). “Steam explosion of wood particles from fibreboard and particle board with indirect control by enzymatic hydrolysis,” *Acta Chimica Slovaca* 12(2), 185-191. <https://doi.org/10.2478/acs-2019-0026>
- Pažitný, A., Russ, A., Boháček, Š., Stankovská, M., Ihnát, V., and Šutý, Š. (2020). “Effect of steam explosion on enzymatic hydrolysis of various parts of poplar tree,” *Wood Res* 65, 579-590. <https://doi.org/10.37763/wr.1336-4561/65.4.579590>
- Pielhop, T., Amgarten, J., von Rohr, P. R., and Studer, M. H. (2016). “Steam explosion pretreatment of softwood: the effect of the explosive decompression on enzymatic digestibility,” *Biotechnology for Biofuels* 9(1), article 152. <https://doi.org/10.1186/s13068-016-0567-1>
- Qing, Q., Yang, B., and Wyman, C. E. (2010). “Impact of surfactants on pretreatment of corn stover,” *Bioresource Technology* 101(15), 5941-5951. <https://doi.org/10.1016/j.biortech.2010.03.003>
- Sharma, R., Palled, V., Sharma-Shivappa, R. R., and Osborne, J. (2013). “Potential of potassium hydroxide pretreatment of switchgrass for fermentable sugar production,” *Applied Biochemistry and Biotechnology* 169(3), 761-772. <https://doi.org/10.1007/s12010-012-0009-x>
- Shimizu, K., Sudo, K., Ono, H., Ishihara, M., Fujii, T., and Hishiyama, S. (1998). “Integrated process for total utilization of wood components by steam-explosion pretreatment,” *Biomass and Bioenergy* 14(3), 195-203. [https://doi.org/10.1016/S0961-9534\(97\)10044-7](https://doi.org/10.1016/S0961-9534(97)10044-7)

- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008). *Determination of Structural Carbohydrates and Lignin in Biomass* (NREL/TP-510-42618), National Renewable Energy Laboratory, Golden, CO, USA.
- Song, K., Chu, Q., Hu, J., Bu, Q., Li, F., Chen, X., and Shi, A. (2019). “Two-stage alkali-oxygen pretreatment capable of improving biomass saccharification for bioethanol production and enabling lignin valorization *via* adsorbents for heavy metal ions under the biorefinery concept,” *Bioresour Technol* 276, 161-169. <https://doi.org/10.1016/j.biortech.2018.12.107>
- Takkellapati, S., Li, T., and Gonzalez, M. A. (2018). “An overview of biorefinery-derived platform chemicals from a cellulose and hemicellulose biorefinery,” *Clean Technologies and Environmental Policy* 20(7), 1615-1630. <https://doi.org/10.1007/s10098-018-1568-5>
- TAPPI T222 om-11 (2011). “Acid-insoluble lignin in wood and pulp,” TAPPI Press, Atlanta, GA, USA.
- Tian, D., Chandra, R. P., Lee, J. S., Lu, C., and Saddler, J. N. (2017). “A comparison of various lignin-extraction methods to enhance the accessibility and ease of enzymatic hydrolysis of the cellulosic component of steam-pretreated poplar,” *Biotechnology for Biofuels* 10(1), article 157. <https://doi.org/10.1186/s13068-017-0846-5>
- Valentine, S. V. (2011). “Emerging symbiosis: Renewable energy and energy security,” *Renewable and Sustainable Energy Reviews* 15(9), 4572-4578. <https://doi.org/10.1016/j.rser.2011.07.095>
- Wang, B., Yang, G., Wang, Q., Liu, S., Chen, J., and Fang, G. (2020). “A new surfactant assisted acid prehydrolysis process for enhancing biomass pretreatment,” *Cellulose* 27(4), 2149-2160. <https://doi.org/10.1007/s10570-019-02950-8>
- Wijeyekoon, S. L., and Vaidya, A. A. (2021). “Woody biomass as a potential feedstock for fermentative gaseous biofuel production,” *World Journal of Microbiology and Biotechnology* 37(8), article 134. <https://doi.org/10.1007/s11274-021-03102-6>
- Xu, L., Zhang, S. J., Zhong, C., Li, B. Z., and Yuan, Y. J. (2020). “Alkali-based pretreatment-facilitated lignin valorization: A review,” *Industrial & Engineering Chemistry Research* 59(39), 16923-16938. <https://doi.org/10.1021/acs.iecr.0c01456>

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