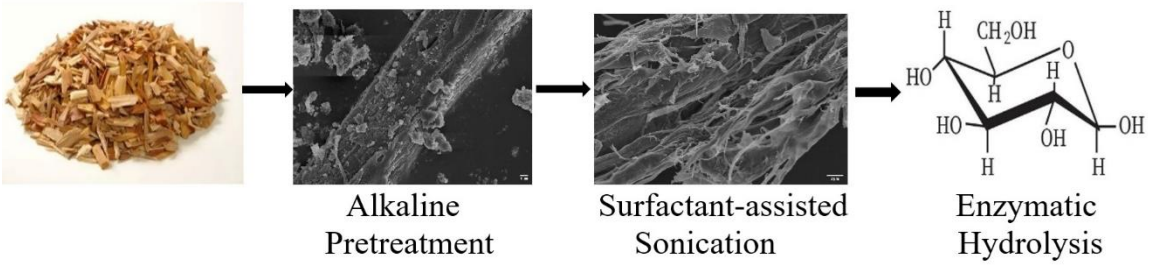
# Robust Pretreatment in Combination with Ultrasound-Assisted Surfactant to Improve the Enzymatic Hydrolysis of *Eucalyptus pellita*

Soodeh Zhand,[a] Bong Suk Yang,[b] Kyu-Young Kang,[c] and Myung-Joon Jeong [d,*]

*DOI: 10.15376/biores.19.2.2244-2271*

**Corresponding author: mjeong@jbnu.ac.kr*

## GRAPHICAL ABSTRACT



# Robust Pretreatment in Combination with Ultrasound-Assisted Surfactant to Improve the Enzymatic Hydrolysis of *Eucalyptus pellita*

Soodeh Zhand,[a] Bong Suk Yang,[b] Kyu-Young Kang,[c] and Myung-Joon Jeong [d,*]

Alkaline pretreatment stands out as a valuable strategy in biomass conversion to overcome the recalcitrance of biomass by removing lignin and a part of hemicellulose. This enhances enzyme accessibility and promotes saccharification. However, increasing the alkaline concentration to enhance the delignification and improve glucose yield together presents inherent limitations. In this experiment the amount of delignification and glucose yield resulting from surfactant associated with sonication during mild alkaline pretreatment of *Eucalyptus pellita* was investigated. Also, the effect of various factors (sonication temperature and surfactant immersion time) on delignification and glucose yield were examined. The results demonstrated that surfactant associated with sonication pretreatment could overcome the limitation of alkaline pretreatment and could increase the amount of delignification alongside enzymatic hydrolysis glucose yield of *Eucalyptus pellita* wood by around 90%. The findings indicated that surfactant-assisted with sonication during mild alkaline pretreatment of *Eucalyptus pellita* (*i.e.,* hardwood) could be recommended as a supporting pretreatment method for the production of monomeric sugars.

*DOI: 10.15376/biores.19.2.2244-2271*

*Keywords: Alkaline pretreatment; Sonication; Surfactant; Enzymatic hydrolysis*

*Contact information: a: Department of Wood Science and Technology, Jeonbuk National University, Jeonju 54896, Republic of Korea; b: Postdoctoral Researcher, Department of Biological and Environmental Science, College of Life Science and Biotechnology, Dongguk University, Seoul, Republic of Korea; c: Professor, Department of Biological and Environmental Science, College of Life Science and Biotechnology, Dongguk University, Seoul, Republic of Korea. d: Associate Professor, Department of Wood Science and Technology, Jeonbuk National University, Jeonju 54896, Republic of Korea;*
** Corresponding author: mjeong@jbnu.ac.kr*

## INTRODUCTION

Biorefinery involves the generation of value-added compounds using biomass, encompassing a broad spectrum from biomaterials to chemical compounds. This process has garnered significant attention within the chemical industry (Cherubini and Ulgiati 2010). In recent decades, the widespread utilization of biomass in generating a diverse range of enhanced-value products has opened up novel possibilities (Cherubini and Ulgiati 2010). Biorefineries typically pursue three primary goals. The initial aim is to guarantee energy security in nations, whether developed or developing, where a substantial quantity of biomass is generated (Martinez-Hernandez *et al*. 2013). The secondary aim involves mitigating environmental impacts and chemical production through the utilization of renewable resources and processes. This entails minimizing waste generation and curbing emissions of greenhouse gases. The tertiary goal is to enhance the quality of life within societies engaged in the supply chain of these products (Coppola *et al*. 2021).

Utilizing lignocellulose as a raw material for bioethanol production offers a sustainable alternative to diminish reliance on fossil fuels and alleviate energy demands (Broda *et al*. 2022). Biomass rich in syringyl units within the lignocellulosic structure exhibits increased susceptibility to delignification at elevated pH levels. Consequently, hardwoods, characterized by the presence of both syringyl and guaiacyl units, prove to be more suitable for alkaline pretreatment compared to softwoods (Lehto and Alén 2013). In terms of lignin size and structure, hardwood has lignin of lower degree of polymerization (DP) and more methoxyl groups than softwood (Brown 2003). For this reason, the authors chose to apply *Eucalyptus pellita* a hard wood as a raw material for alkaline pretreatment. *Eucalyptus pellita* exhibits favorable traits such as rapid growth and exceptional resistance to diseases and pests (Fadwati *et al*. 2023).

A variety of physical, biological, chemical, and thermal pretreatments and their combinations have been studied (Kim and Lee 2006; Kim *et al*. 2008; Kim *et al*. 2011). The main aim of pretreatment is to remove hemicelluloses and/or lignin, thereby reducing the recalcitrance of lignocellulosic materials. Since the lignocellulosic biomass show recalcitrance to cellulase, pretreatment of lignocellulosic materials is crucial because of the inherent resistance of untreated lignocellulosic substances to enzymes. Efficient pretreatment is crucial to enhance enzyme access to plant polysaccharides (Li *et al*. 2022). Pretreatment methods are necessary to disrupt lignin, which binds cellulose, perturbing the crystalline structure of cellulose, and augmenting its surface area, thereby enhancing hydrolytic efficiency and sugar yield (Wu *et al*. 2023). Alkaline pretreatment is the predominant method for removing lignin and hemicelluloses from lignocellulosic materials. This process causes lignocellulosic biomass to swell and the fibers to separate. During alkaline pretreatment, a critical reaction occurs, leading to the cleavage of ester linkages in both hemicelluloses and lignin (Agbor *et al*. 2011). The bonds between the lignin and carbohydrates are broken, and some lignin is solubilized (Balat *et al*. 2008; Hendriks and Zeeman 2009; Galbe and Zacchi 2012). Among various alkaline compounds, sodium carbonate is more cost-effective than sodium hydroxide, being 4 to 6 times cheaper and easier to recover. Additionally, sodium hydroxide is comparatively economical, being 3 to 6 times cheaper than calcium hydroxide (Krishania *et al*. 2013). Previous alkaline pretreatments usually focused on sodium hydroxide as alkali liquor (de Carvalho *et al*. 2016; Guigou *et al*. 2019). In the authors' previous study, a dilute alkaline pretreatment (sodium carbonate and calcium hydroxide mixture) was evaluated for saccharification of extruded *Eucalyptus* wood. The results showed that the glucose conversion by the enzyme was improved by approximately 14% for every 10% increase in delignification rate (Zhand *et al*. 2020). Alkaline pretreatment plays a crucial role in delignifying biomass, enhancing its enzymatic digestibility. The cleavage of alkyl aryl linkages in lignin occurs readily in alkaline conditions, with the reaction primarily influenced by the bond structure. Importantly, the reaction is not significantly affected by [OH−] as long as the pH remains above 10 (Park and Kim 2012).

Over the past few decades, various researchers have consistently demonstrated the adverse impact of lignin on cellulose hydrolysis (Nakagame *et al*. 2011; Pareek *et al*. 2013; Guo *et al*. 2014). Enzymatic hydrolysis encounters difficulties in the presence of lignin, as it significantly absorbs cellulase, impeding the access of enzymes to cellulose and hindering their enzymatic activity (Lu *et al*. 2002). Additionally, lignin restricts enzyme entry to cellulose and hemicellulose, resulting in prolonged reaction times for conversions (Sutcliffe 1986; Lu *et al*. 2002; Wyman 2007). As a result, an effective method to mitigate

the detrimental impact of lignin on enzymatic hydrolysis involves either eliminating or altering the lignin content. It is proposed that during pretreatment, lignin is released into a solution and, in subsequent reactions, it forms compounds with limited solubility that precipitate onto the fiber surface (Liu and Wyman 2003; Wyman 2007). These lignin deposits interfere with enzyme activity, leading to nonproductive enzyme adsorption (Donohoe *et al*. 2008). The proposed mechanisms involve nonproductive binding of enzymes with lignin, resulting in the physical obstruction of enzymes by lignin. Cellulases are suggested to adhere to lignin through hydrophobic interactions, electrostatic forces, and hydrogen bonding interactions (Nakagame *et al*. 2011; Pareek *et al*. 2013). The utilization of nonionic surfactants proves to be an effective strategy for minimizing the nonproductive binding of enzymes to lignin, enhancing biomass hydrolysis yields significantly. Considering saccharification's considerable expense, there is a necessity to implement modifications aimed at cost reduction (Eriksson *et al*. 2002).

By means of introducing surfactants, particularly nonionic ones, post-pretreatment has the potential to enhance enzymatic hydrolysis or decrease the required enzyme quantity for achieving a specific conversion (Kaar and Holtzapple 2000; Eriksson *et al*. 2002; Qing *et al*. 2010). Surfactants enhance digestibility by modifying substrate structure for improved accessibility (Kaar and Holtzapple 1998), stabilizing enzymes to prevent denaturation during hydrolysis (Kim *et al*. 1982), promoting positive interactions between substrates and enzymes (Kaar and Holtzapple 1998; Eriksson *et al*. 2002), and mitigating non-productive enzyme adsorption (Eriksson *et al*. 2002). To meet the criteria for an ideal pretreatment strategy, several conditions should be met. They include being economical, optimally deconstructing lignin, effective for various lignocellulosic substrates, causing minimal glucan loss, avoiding corrosive chemical reagents, reducing waste generation and environmental pollution, and generating low levels of fermentation inhibitors, ultrasonic irradiation has emerged as a pollution-free pretreatment approach (Gaudino *et al*. 2022).

In recent years, the growing concern for environmental impact has led to increased interest in pollution-free pretreatment approaches. One such method gaining widespread use in various industrial processes is ultrasonic irradiation. This physicochemical treatment involves the release of energy through acoustic waves with frequencies greater than 16 to 20 kHz. Ultrasonic irradiation is recognized for its ability to achieve pretreatment without the use of chemical reagents, aligning with the goal of minimizing pollution and environmental harm (Onu Olughu *et al*. 2021). Utilizing ultrasound during pretreatment induces cavitation in the aqueous medium. As the cavitation bubbles collapse, water decomposes into radicals, facilitating the cleavage of lignin linkages and the xylan meshwork. The cavitation process generates microbubbles at multiple nucleation sites in the fluid. The implosion and collapse of these bubbles result in the release of intense shock waves that propagate throughout the medium (Yaldagard *et al*. 2008).

To overcome the recalcitrance of the lignocellulosic material, prior enzymatic hydrolysis alkaline pretreatment was done. Merely applying alkaline pretreatment will not result in the desired glucose yield due to lower delignification. To overcome the obstacle, ultrasound assisted surfactant and enzymatic hydrolysis of *Eucalyptus* for the production of bio glucose was investigated. Comparing to the experiment done by Elalami *et al*. (2022), the highest glucose yield was 86% obtained by ultrasonication coupled to alkaline pretreatment of bean straw. Cheng *et al*. (2022) demonstrated that surfactant-assisted alkaline pretreatment (SAP) with elevated surfactant concentrations (1% vs. 0.5%) increased sugar yields. SAP assisted by 1% surfactant concentration showed improved results. In the study by Ziaei-Rad *et al*. (2023), a robust pretreatment technique based on

enzymatic hydrolysis was investigated, evaluating the saccharification yield, including the amount of glucose and xylose produced.

In this study, alkaline pretreatment with sodium carbonate and calcium hydroxide was applied alongside surfactant associated sonication to increase delignification and glucose yield. The research could offer valuable information on utilizing hardwood substrates efficiently for the production of bioethanol.

## EXPERIMENTAL

### Materials

*Wood preparation*

*Eucalyptus pellita* chips were imported from Indonesia with dimension of 2.5 to 3 cm in width and 1 to 3 cm height. The chips underwent physical pretreatment using a twin extruder at the Korea Research Institute of Science and Technology (KRIST) for use as an experimental substrate. The process involved the use of *Eucalyptus* wood chips, extruded with a twin-screw extruder (Baker Perkins, MPC30, L / D = 13). Following this, a mixture of dry biomass and distilled water in a 1:10 ratio was subjected to twin-screw extrusion treatment. Subsequently, an equal amount of distilled water was added, and the resulting material was dehydrated using a filter press for 15 min. The recovered sample was utilized for pretreatment and saccharification without undergoing any specific drying procedures. The moisture content of the sample subjected to twin-screw extrusion was 56.3%. Table 1 shows the chemical composition of *Eucalyptus* wood.

**Table 1.** Chemical Composition of Eucalyptus Wood

| | | |
|---|---|---|
| Lignin Content (%) | Acid Insoluble Lignin | 32.3±0.35 |
| | Acid soluble lignin | 1.3±0.42 |
| Extractives (%) | 0.8±0.06 | |
| Ash (%) | 0.2±0.05 | |
| Natural sugar analysis (%) | Glucose | 52.6±0.40 |
| | Xylose | 10.6±0.21 |
| | Mannose | 0.3±0.04 |
| | Galactose | 1.4±0.32 |
| | Arabinose | 0.5±0.02 |

*Alkaline pretreatment*

Physically pretreated *Eucalyptus pellita* with twin extruder used for mild alkaline pretreatment process with sodium carbonate ($Na_2CO_3$) and calcium hydroxide ($Ca(OH)_2$) as chemicals. The materials were procured from Sigma-Aldrich®, and the experiment utilized the laboratory's highest-grade 18.2 MΩ deionized water (DI).

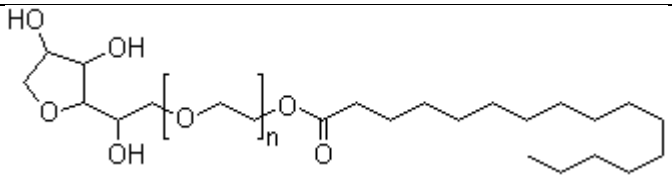
Various concentrations of sodium carbonate (0.4, and 0.5 M) and calcium hydroxide (0.25, 0.4, and 0.5 M) were examined and the pH of the mixture were averaged from 12.98 to 13.01. To create a 0.5 M sodium carbonate solution, 53 g of sodium carbonate dissolved in 500 mL of DI, combined with 0.74 g (0.5 M) of calcium hydroxide and 10 mL of DI. Similarly, for preparation of 0.4 M sodium carbonate solution, 42.3 g of sodium carbonate dissolved in 500 mL DI, combined with 0.59 g (0.4 M) of calcium hydroxide and 10 mL of DI. Also, for preparation of 0.25 M of calcium hydroxide 0.37 g of calcium hydroxide combined with 10 mL of DI. The experiment was conducted at a liquid (L): lignocellulosic material (W) ratio of 10:1 in a 500 ml closed reactor, stirring for

30 min at 180 °C. Following alkaline pretreatment, the samples underwent 3 to 4 washes with DI water to achieve a pH of 6.5.

*Ultrasound assisted surfactant pretreatment*

Ultrasound assisted surfactant treatment of *Eucalyptus* was carried out in a glass beaker that was sealed with aluminum foil to save the heat generated from sonication process. The slurry weight reached to 100 g and 3% (w/w) polyoxyethylene sorbitan monopalmitate (Tween 40®) surfactant was added in to slurry and set the pH to 5.0 and immersed for 1 h and 24 h, after this section the reactors were sealed with aluminum foil and sonication under ultrasonic device (Sonics Vibra Cell model VCX 750 W 20kHz) with a 13 mm probe sonicator which was 5 cm immersed deep into the slurry was done. The ultrasonic irradiation at the 40% amplitude was transferred through a titanium cylindrical horn, introduced into the solution. The horn had a diameter of 1.1 cm, and the probe temperatures were varied (50, 70, and 90 °C). The variables used in this study were alkaline concentration, surfactant immersion time, and sonication temperature. After pretreatment, the samples were filtrated and washed with DI water to get ready for enzymatic hydrolysis.

**Table 2.** Tween-40 Properties

| **Product Name** | **Tween 40®** |
|---|---|
| Type | Non-ionic |
| Name | Polyoxyethylene Sorbitan Monopalmitate |
| Molecular Formula | $C_{22}H_{42}O_6.(C_2H_4O)_n$ |
| Chemical structure |  |
| Critical Micelle Concentration | 0.027mM (20-25°C) |
| Hydrophilic-lipophilic balance (HLB) value | 15.6 |

*Enzymes hydrolysis assisted surfactant*

Pretreated samples were selected for enzymatic hydrolysis. Enzymatic hydrolysis experiments utilized Novozymes Chemical Company's commercial enzyme solutions, Cellic® CTec2 and HTec2, generously provided by KRIST. The process involved placing 1 g of oven-dried pretreated fibers in a solution containing 2.5 mL sodium citrate buffer at pH 5.5, along with a 1.3 mL sodium azide 1% solution (biocide). Additionally, 0.1 mL of a Cellic® CTec2 and HTec2 mixture (6.480:0.725) was added, adjusting the final volume to 40 g with DI water.

The suspension's pH was set to 5.5. The reaction occurred in a shaking incubator (150 rpm) at 50 °C for 72 h. Sampling every 3 h involved placing 0.5 mL of the sample in a micro-tube, and the reaction was stopped by exposing the micro-tube to a hot plate at 100 °C for 5 min. The resulting mixture underwent vacuum filtration, and glucose concentration analysis was performed.

*Chemical composition analysis*

The acid-insoluble lignin content in the raw materials was assessed following the TAPPI T222 om-02 test method. Subsequently, the primary and secondary acid hydrolyzed samples underwent evaluation for acid insoluble lignin by analyzing the filtrate. The analysis of neutral sugar (glucose) from the filtrate was conducted using the Bio-LC system (ICS-3000, Dionex, USA). This analysis adhered to the NREL/TP-510-42618 protocol for determining structural carbohydrates and lignin in biomass.

*Field emission scanning electron microscope (FE-SEM) analyses*

In this case, samples were vacuumed and freeze-dried prior to FE-SEM. Specimen preparation prior to SEM analyses was needed; in this case a Leica EM ACE 200 was used to produce homogenous and conductive carbon coatings for SEM analysis. In electron microscopy, the application of sample coatings is essential for enhancing or enabling sample imaging. The process involves depositing a conductive metal layer on the sample, preventing charging, minimizing thermal damage, and enhancing the secondary electron signal crucial for topographic analysis in the Scanning Electron Microscope (SEM). The Zeiss Supra 40VP SEM at JBNU delivers high-resolution surface imaging with a three-dimensional appearance. It features a field emission cathode as the electron source and is equipped with three distinct detectors (SE2, InLens, STEM).

All the data were analyzed and graphs were made by Origin Pro 2023 ver. Software.

## RESULTS AND DISCUSSION

### Effect of Alkaline Pretreatment

Sodium carbonate pretreatment can remove lignin and various uronic acid substitutes in hemicellulose but cause less degradation of cellulose than acid or hydrothermal pretreatments. Additionally, it has the potential to address significant operational challenges associated with traditional alkaline pretreatment methods, including issues such as corrosion, extensive neutralization requirements, and environmental hazards (Kim *et al*. 2011; Kim and Kim 2014). Compared to aqueous sodium hydroxide pretreatment, calcium hydroxide (lime) pretreatment offers cost advantages and environmental benefits due to its ease of recovery through reaction with carbon dioxide (Suslick 1989). Also, it may result in changes in structural features such as acetylation, crystallization, and lignification (Kim and Lee 2006). Based on these findings, the decision was made to employ a combination of mild alkaline substances, namely sodium carbonate and calcium hydroxide, for alkaline pretreatment. The pretreatment process was conducted at a temperature of 180 °C at the H factor of 1200 for a duration of 30 min. This condition was chosen due to the results of the authors' previous work on the optimum temperature and time to reach the highest glucose yield was alkaline pretreatment at 180°C and 30 min (Zhand *et al*. 2020). The sodium hydroxide generated during this process plays a crucial role in disrupting the bonds within the Lignin Carbohydrate Complex (LCC) linkage, specifically targeting and breaking the ether and ester bonds in the LCC structure. Furthermore, sodium hydroxide proves effective in breaking the ester and carbon-to-carbon (C–C) bonds present in lignin molecules, such as ferulic acid (Wang *et al*. 2020a). Equation 1 contains the delignification calculation,

$$\text{Delignification} = \frac{(100 - L_c) \times C}{L_r} \quad (1)$$

where $C$ is the biomass content after pretreatment (g), $L_p$ is the percent of lignin content in pretreated sample (%), and $L_r$ is the percent of lignin content in raw *Eucalyptus* (%).

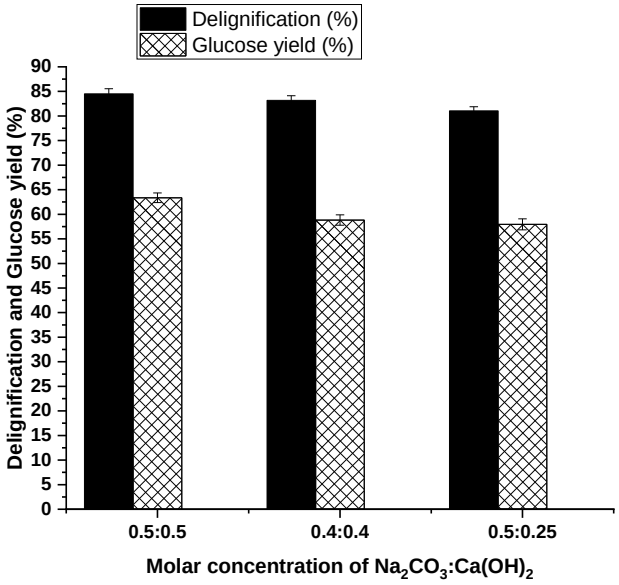



**Fig. 1.** Comparison between glucose yield by enzymatic hydrolysis of alkaline pretreated samples and delignification by alkaline pretreatment

In this work mild alkaline conditions for pretreating of eucalyptus were applied.

$$Na_2CO_3 + Ca(OH)_2 \rightarrow 2NaOH + CaCO_3 \quad (2)$$

The reaction is used in the manufacturing of NaOH, and the pH of the solution was found to be greater than 14, indicating a high concentration of NaOH.

This reaction is an example of a double displacement reaction, also known as a metathesis reaction. In this type of reaction, the cations and anions switch between two reactants to form new products (Simoni *et al*. 2022).

$$Na_2CO_3 + H_2O \rightleftharpoons 2(Na^+OH^-) + CO_2 \quad (3)$$

Sodium carbonate dissolves in water and produces sodium hydroxide. The dissociation of sodium carbonate yields sodium ions ($Na^+$) and hydroxide ions ($OH^-$), and the latter combines with another sodium ion to form sodium hydroxide. Simultaneously, carbon dioxide ($CO_2$) is liberated (Toan *et al*. 2019).

The aim of different concentrations of sodium carbonate and calcium hydroxide was to determine the optimal concentration under a limited alkaline assumption. Specifically, for the ratio of sodium carbonate and calcium hydroxide 0.5/0.25 M, the reduction in calcium hydroxide concentration by half had a strategic purpose. The process involves a reversible reaction represented by Eq (3). By reducing the concentration of calcium hydroxide, the equilibrium can shift towards the forward reaction, promoting the production of ($OH^-$) ions in the medium. The decrease in calcium hydroxide concentration facilitates the forward reaction, thereby leading to an increased generation of hydroxide ions ($OH^-$) in the solution. Importantly, this shift helps to eliminate the precipitation of calcium carbonate. The reversible reaction tends to move forward, minimizing the formation of calcium carbonate. However, the experimental results indicated that halving the concentration of calcium hydroxide led to a tendency for the reaction to move

backward. This suggests that, in this specific reaction, the reduction in calcium hydroxide concentration did not favor the desired increase in ($OH^-$) ions (Woo *et al*. 2020; Ahenkorah *et al*. 2021).

As was shown in the authors' previous experiment (Zhand *et al*. 2020), the highest amount of glucose yield was 60.3% after alkaline pretreatment with the combination of (0.6/0. 5 M) of sodium carbonate and calcium hydroxide with the same time and temperature of this experiment. Compared to our current work, the highest amount of glucose yield after alkaline pretreatment was 63.3% for the combination of (0.5/0. 5 M) of sodium carbonate and calcium hydroxide.

By applying low concentrations of sodium carbonate and calcium hydroxide (0.5 /0.25M), delignification reached 81.0%. This can be compared with the authors' previous work (Zhand *et al*. 2020) at the same reaction time and temperature with (0.6/0. 5 M) of sodium carbonate and calcium hydroxide, where the delignification was 87.4%. In the cited work, two equal concentrations of sodium carbonate and calcium hydroxide (0.4/0.4M and 0.5/0.5M) were applied to examine differences in equal concentrations. It was shown that applying (0.5/0.5M) concentrations of sodium carbonate and calcium hydroxide led to 84.4% delignification, which is the highest amount of delignification. In comparison with other concentrations of sodium carbonate and calcium hydroxide, the delignification with (0.4/0.4M) was 1.3% less than (0.5/0.5M). Also, the delignification of unequal concentration (0.5/0.25M) was the lowest, which was determined by applying equal concentrations of sodium carbonate and calcium hydroxide the higher delignification can reach. The aim of using mild alkaline was that mild alkaline conditions are typically achieved using lower concentrations of alkali results in partial removal of lignin and hemicellulose, while strong alkaline solution facilitates more extensive removal of lignin and hemicellulose (Kim *et al*. 2016). Thorough delignification leads to a higher degree of wood component breakdown. Such conditions are often chosen for processes requiring greater modification and extraction of wood components (Kim *et al*. 2016). Oriez *et al.* (2020) have shown that cellulose saccharification and fermentation into ethanol giving higher yields (60 to 80% solubilization for lignin and hemicelluloses) can be achieved with mild alkaline pretreatment in comparison to mineral acid fractionation. Moreover, unlike acid fractionation, mild alkaline fractionation does not hydrolyze the sugar polymers, which can be beneficial for certain applications (Oriez *et al*. 2020).

Bali *et al*. (2015) investigated the impacts of various alkaline pretreatments on the structural characteristics and accessibility of cellulose in *Populus*. The most significant enhancement in cellulose accessibility occurred with mild sodium hydroxide treatment, with subsequent notable effects observed in methods involving ammonia soaking and lime (Bali *et al*. 2015). Jiang *et al*. (2020) examined the impact of sodium hydroxide and calcium hydroxide pretreatments on giant reed, focusing on enzymatic digestibility and energy conversion efficiency. Sodium hydroxide pretreatment demonstrated superior removal of lignocellulosic biomass and lignin compared to calcium hydroxide pretreatment at equivalent loading levels (Jiang *et al*. 2020).

## Surfactant Effect

General consensus acknowledges that nonionic surfactants universally enhance hydrolysis, establishing them as the most efficient surfactants for cellulose conversion (Ooshima *et al*. 1986; Kaya *et al*. 1995). The surface activity of surfactants is believed to result in a decreased interaction between enzymes and the air-liquid interface (Kim *et al*. 1982). Nonionic surfactants, such as Tween and PEG series, have been found to be highly

effective in enhancing enzymatic hydrolysis (Wang *et al*. 2020b). They can increase the reaction rate of the enzymatic hydrolysis of cellulose (Rokhati *et al*. 2022). This is achieved by reducing unproductive binding of the enzyme to lignin (Parnthong *et al*. 2018). Nonionic surfactants increase cellulase adsorption and enzymatic hydrolysis of treated lignocelluloses, indicating a positive impact (Wang *et al*. 2020b). The introduction of nonionic surfactants may potentially disrupt or displace some of the intra- and intermolecular hydrogen bonds within lignin molecules, thus facilitating their dissolution (Melro *et al*. 2021). On the other hand, ionic surfactants can have a negative effect on the enzymatic hydrolysis of cellulose. Lee *et al*. (2021) found that the rate of hydrolysis decreased by 36%, 38%, and 50% in the presence of amphoteric, anionic, and cationic surfactants, respectively. This decrease in hydrolysis rate could be due to the surfactant interfering with the enzyme's ability to bind to the cellulose substrate (Lee *et al*. 2021). Therefore, based on the available information, nonionic surfactants seem to be more effective in enhancing the enzymatic hydrolysis of cellulose.

According to these, Tween 40® a non-ionic surfactant, was applied in this experiment. Effect of surfactant on hydrolysis improvement depends on several factors. In this work, the effect of surfactant immersion time was examined relative to the glucose yield and delignification rate.

The enhancement of lignocellulose hydrolysis by surfactants is attributed to their capability to disturb the substrate, leading to increased accessibility of cellulose to enzymes (Kaar and Holtzapple 1998). The present findings regarding enzyme adsorption during hydrolysis in the presence of Tween 40® corroborate this hypothesis. While a more intricate mechanism elucidating the specific role of Tween 40® is not provided, the present results clarify the primary impact of surfactants on enzyme-substrate interactions. The surfactant's influence on lignocellulose hydrolysis can be delineated by the hydrophobic segment of the surfactant binding to lignin through hydrophobic interactions, and the hydrophilic head group, thus preventing non-productive binding of cellulases to lignin (Sánchez-Muñoz *et al*. 2022). The hydrophilic components of nonionic surfactants consist of short ethylene oxide chains. The adsorption of surfactants with ethylene oxide chains onto a lignin surface results in the occupation of the hydrophobic head, leading to the displacement of adsorbed enzymes (Kim *et al*. 1982; Malmsten and Van Alstine 1996). The extent of unproductive enzyme adsorption onto lignin is significantly influenced by the presence of lignin in the medium. Higher levels of delignification lead to reduced exposure, resulting in diminished unproductive binding of enzymes to lignin (Wu *et al*. 2023).

When samples were subjected to 0.5/0.25M of $Na_2CO_3$/Ca $(OH)_2$ pretreatment, the highest amount of delignification was (86.6%) for the samples that were immersed in surfactant for 1 h. With the same situation but higher surfactant immersion time (24 h), the delignification increased to 87.8%, which was 1.2% higher. The surfactant Tween-40 was able to increase the delignification rate at high temperature of sonication. The incorporation of surfactants resulted in elevated lignin removal by functioning as agents for delignification (Sindhu *et al*. 2013) through hydrophobic interactions with lignin (Qing *et al*. 2010). The mechanism of surfactant was investigated mainly from the aspects of increasing delignification alongside increasing glucose yield. In principle, increasing cellulase accessibility is achieved by decreasing unproductive adsorption during the interaction of enzyme and substrate. The present results on increasing the amount of delignification by applying nonionic surfactant (Tween-40) well match with the results of Melro *et al*. (2021), which investigated the impact of various surfactants (ionic, cationic, and nonionic) on lignin dissolution. In that work, it was observed that the nonionic

surfactant exhibited a distinct influence. This effect is likely correlated with the quantity of ethylene and hydroxyl groups in the nonionic surfactant, leading to the creation of hydrogen bonds between the ethylene oxide and hydroxyl units of the surfactants and the hydroxyl groups in lignin. These interactions promoted the formation of complexes between lignin and surfactants. As lignin possesses both hydrogen bond donors and acceptor groups, it has the capability to establish intra- and intermolecular hydrogen bonds (Melro *et al*. 2021).

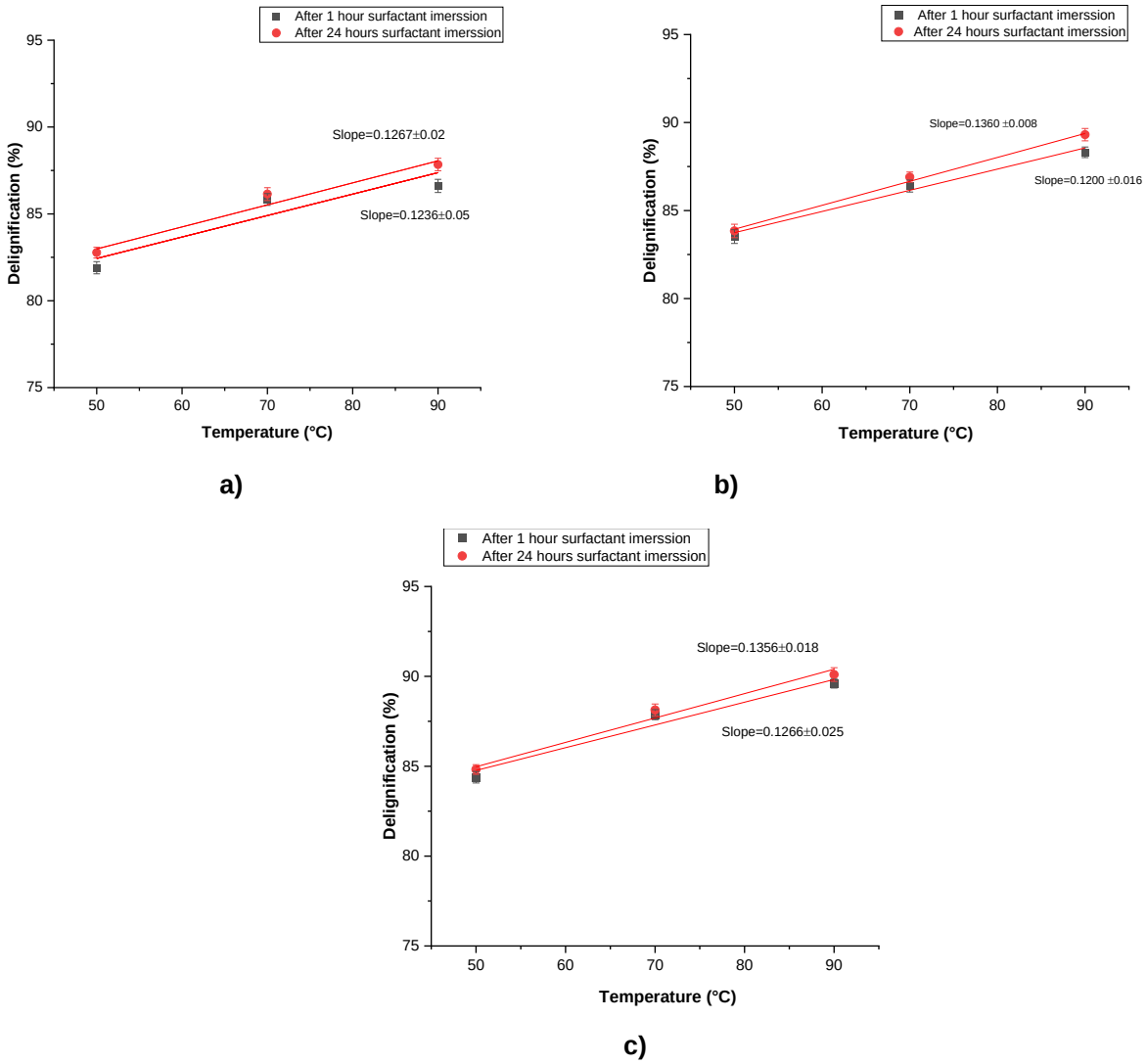



**Fig. 2.** Effect of surfactant immersion time on the rate of delignification for the samples which were subjected to surfactant for 1 h and 24 h. a) Samples were pretreated with the alkaline concentration of $Na_2CO_3$/Ca $(OH)_2$ of 0.5/0.25M, b) 0.4/0.4M, c) 0.5/0. 5M.

Figure 2(b,c) shows that by increasing the alkaline concentration and sonication temperature, delignification reached 89.3% and 90.1%, respectively. Comparison of the results of delignification after alkaline pretreatment (Fig. 1) and after surfactant-assisted sonication (Fig. 2), demonstrated that surfactant associated sonication could increase the delignification by 5.6% to 6.8% more than the samples were pretreated only by alkaline $Na_2CO_3$/Ca $(OH)_2$. The results also showed that there was a 0.5 to 1.2% increase in

delignification rate by immersing the surfactant for 24 h.

In summary, immersion of surfactant in slurry before the enzymatic hydrolysis can increase delignification. The decreased surface tension of the surfactant will increase lignin removal and crystalline cellulose deconstruction (Luo *et al*. 2014). Zhang *et al*. (2020) employed varying concentrations of NaOH (ranging from 0% to 10%). NaOH-catalyzed organosolv pretreatment was implemented to enhance the enzymatic efficacy of sugarcane bagasse. Additionally, the impact of Tween-80 on enzymatic hydrolysis following NaOH-catalyzed organosolv pretreatment was investigated, resulting in the attainment of the highest glucose yield at 95.1% after 24 h (Zhang *et al*. 2020). Although delignification increased by increasing the immersion time of surfactant, the effect of sonication temperature on delignification was more significant.

## Effect of Ultrasonic Pretreatment

Sonication involved the application of sound waves as a physicochemical treatment method, delivering energy to the system. Ultrasound, characterized by frequencies exceeding 16-20 kHz, is transmitted to a fluid through a sonic horn. The sonic horn's precise frequency causes it to oscillate along its vertical axis. To convey this acceleration and sonic energy to the sonication medium, it is essential to immerse the horn's tip in the medium (Chisti 2003). The utilization of ultrasound treatment induces the formation of small cavitation bubbles on a microscopic scale. These bubbles effectively break down the cellulose and hemicellulose components, thereby enhancing the accessibility of cellulose-degrading enzymes necessary for the hydrolysis of these complex carbohydrates (Kumar and Sharma 2017). The application of ultrasonic irradiation induces sonochemical and mechano-acoustic effects, leading to the delignification and surface erosion of lignocellulosic biomass throughout the treatment process. (Onu Olughu *et al*. 2021). Sonication induces homolysis of lignin–carbohydrate bonds, liberating lignin and hemicellulose. Aromatic rings within lignin undergo α-position cleavage of C–C bonds, and susceptibility of C–H bonds to disruption occurs. This bond disruption results in the generation of macroradicals. The collaboration of these macroradicals with radicals such as ˙H and ˙OH, produced through cavitation, triggers the depolymerization of lignocellulosic material (García *et al*. 2012).

There would be an optimal temperature for the desirable ultrasonic effect. Raising the temperature accelerates the chemical reactions involved in biomass pretreatment. However, an excessively high temperature can have adverse effects and impede the overall process. Collapsing the bubbles during the compressive section may be slowed by increasing the temperature. High temperature reduces the solubility of gases in a liquid which is undesirable in cavitation and adversely effects the sonication process (Prabhu *et al*. 2004; Montalbo-Lomboy *et al*. 2010). As the temperature increased the amount of delignification increased up to (90.1%) the reason of higher delignification is maybe higher temperature which leads to decreasing particle size of the slurry and using the heat generated from sonication caused the slurry work as a hot water pretreatment which causes swelling the fibers alongside vibration in sonication causes more delignification. But, increasing the temperature by more than 50 °C led to a decrease in glucose yield.

In this study, sonication was subjected to alkali pretreated samples, augmented with 3% (w/w) nonionic surfactant Twin-40. The investigation involved applying different ultrasound treatment temperatures during raw material fractionation using surfactant, varying immersion times.

Ultrasound-assisted depolymerization of lignin has been studied, with factors such

as catalyst types, reaction temperatures, reaction times, reaction solvents, and ultrasound frequencies being investigated to improve the yields and characteristics of various depolymerization products of lignin. The results showed that ultrasound acoustic cavitation could enhance the depolymerization of lignin, thus greatly enhancing the production of liquid fuels (Du *et al*. 2020). Prabhudesai *et al*. employed an ultrasonic pretreatment of the pine wood and the degree of delignification and the selectivity enhanced towards lignin monomers significantly (Prabhudesai *et al*. 2023). Olguin-Maciel *et al.* did a study on ultrasound pretreatment of non-conventional starch at different sonication temperature conditions (45, 70, and 90 °C). The results showed that the effect of ultrasound is more noticeable at low temperatures (Olguin-Maciel *et al*. 2022). Similarly, Zhu and Li worked on quinoa flour functionality using different sonication temperature and sonication effect was more evitable at lower temperature (Zhu and Li 2019). This could be explained by the possibility that at elevated temperatures, the energy conveyed diminishes, leading to a reduction in cavitation (Kardos and Luche 2001). Sun *et al*. conducted an experiment to optimize the ultrasonication temperature for the production of xylooligosaccharides from corn cob. They found that changes in temperature can affect the speed of molecular thermal motion and the cavitation effect, which are crucial for enzymatic action (Sun *et al*. 2023). The objective of varying sonication temperature in ultrasound assisted alkaline pretreatment was to achieve the optimum temperature for sonication effect and examine the effect of sonication temperature on enhancement of both delignification and glucose yield.

## Enzymatic Hydrolysis

Alkaline pretreatment effectively addresses chemical and physical impediments, rendering polysaccharides readily accessible for enzymatic digestion. Factors influencing cellulose accessibility encompass cellulose crystallinity, the composition and distribution of lignin and hemicelluloses, and the available surface area, enzymatic hydrolysis was conducted by Karimi *et al*. to explore the optimal conditions for pretreated eucalyptus (Karimi and Taherzadeh 2016). The glucose concentration data obtained from the enzymatic hydrolysis of the pretreated samples is visually depicted in Fig. 3.

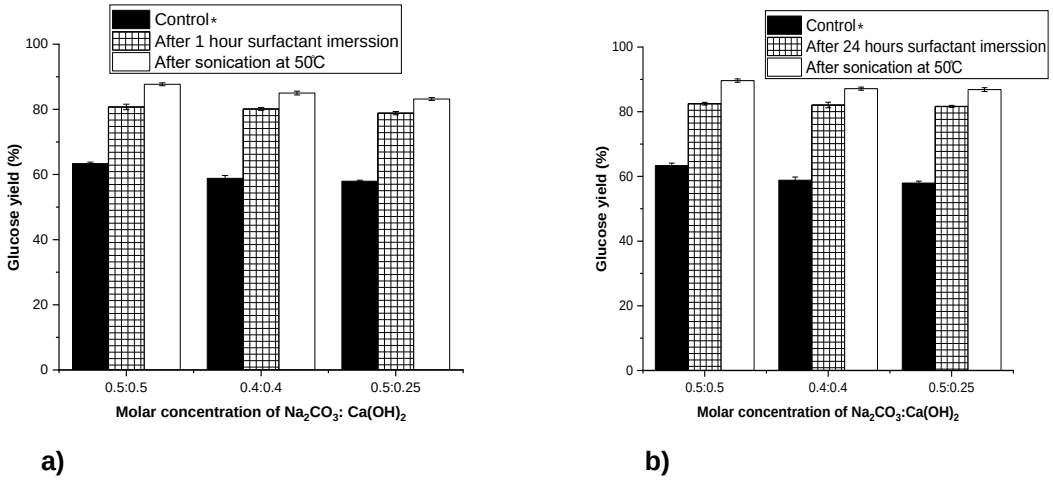



**Fig. 3.** The effect of 3% surfactant immersed for 1 h and 24 h without sonication and enzymatic hydrolysis on glucose yield. a) surfactant immersion for 1 h, b) for 24 h (Control*: the samples without surfactant and without sonication pretreatment).

Surfactants demonstrated a notable enhancement in cellulose enzymatic hydrolysis. Remarkably, their impact was most pronounced in *Eucalyptus* subjected to sodium carbonate and calcium hydroxide treatment, leading to an impressive cellulose conversion increase exceeding 80%. The alkaline pretreatment potentially enhances surfactant adsorption by altering surface properties, such as increased hydrogen bonding capacity. Additionally, it is plausible that the treatment dissolves hemicelluloses associated with or covering lignin, thereby augmenting cellulase accessibility and subsequent cellulose adsorption. Extending the treatment duration may further enhance accessibility, consequently increasing the expected glucose yield (Ghavi *et al*. 2021; Cheng *et al*. 2022). However, further research is imperative to establish a definitive correlation between surfactant effects and substrate characteristics.

To examine the effect of surfactant on glucose yield some samples were not subjected to the sonication pretreatment and after alkaline pretreatment they were immersed in 3% (w/w) surfactant for 1 and 24 h. As the results in Fig. 3a showed the amount of glucose yield after alkaline pretreatment 0.5/0.5 M of $Na_2CO_3$/Ca $(OH)_2$ was 63.3% which increased 17.3% after applying Twin-40 surfactant and immersed for 1h. The glucose yield after applying sonication at 50 °C for 1 h reached 87.7% which is 6.9% increased. This glucose yield after 24 h surfactant immersion (Fig. 3b) was increased 19.1% and reached to 82.5% compare to samples which were immersed 1 h in surfactant 1.7% was increased in glucose yield. After sonication at 50 °C for 1 h the amount of glucose yield for the samples were immersed in Twin-40 for 24 h was increased 7.1% and reached to 89.6% and compare to the same situation but 1 h surfactant immersion time there was 1.9% increase in glucose yield after 24 h immersing surfactant. Generally, surfactant can increase glucose yield up to 21.3% and sonication at 50 °C for 1 h can increase glucose yield up to 7.0%, which these rates increased to 23.7% and 7.1% for samples immersed in twin-40 for 24 h. On the other hand, by applying surfactant (Twin-40) after alkaline pretreatment it's possible to achieve 82.5% glucose yield, which this amount will reach to 89.6% by subjecting the pretreated samples to sonication pretreatment.

As the results showed, there is a direct relationship between delignification and glucose yield after enzymatic hydrolysis for the samples which were immersed in surfactant for 1 h and 24 h. By increasing the temperature in the sonication section, more disruption of the wood structure will occur which causes increasing in delignification. But the amount of glucose yield will decrease by increasing sonication temperature. A comparison of Figs. 2 and 4 for the samples pretreated with 0.5/0.5M of $Na_2CO_3$/Ca $(OH)_2$ at 24 h surfactant immersion time, illustrated that by increasing the sonication temperature from 50 to 90 °C the amount of glucose conversion after enzymatic hydrolysis decreased by about 15% for each 5% increase in the delignification rate. Also, the results showed that this kind of pretreatment removed 89.6% and 90.1% lignin from eucalyptus after 1- and 24-h immersion with surfactant, respectively (Fig. 3). The rise in sonication process temperature led to an increase in delignification rates but a simultaneous decrease in glucose yield. This phenomenon may be attributed to the enhanced extent of delignification, the solvent becomes saturated with lignin and some lignin reprecipitated on fibers, enhanced unproductive enzyme-lignin binding, and reduced glucose yield. Additionally, despite various mechanisms explaining surfactant effects in reducing non-productive enzyme binding to lignin, it is plausible that lignin interaction is not the sole mechanism for the positive impact of surfactants. It is suggested that surfactants may stabilize enzymes *via* different pathways (Yoon and Robyt 2005). It is possible that

surfactants may have a stabilizing effect on an enzyme/substrate complex (Kristensen *et al*. 2007).

Surfactants play a vital role in the pretreatment and enzymatic hydrolysis of lignocellulosic biomass. They can help to enhance the delignification and saccharification of the biomass, thereby improving the yield of reducing sugars (Cheng *et al*. 2022). According to the experiment done by Cheng *et al*. a strategy for coupling surfactant-assisted alkaline pretreatment with surfactant-assisted enzymatic hydrolysis has been proposed for improving sugar recovery from lignocellulosic biomass and results showed that the surfactant-assisted alkaline pretreatment process with 1% of PEG 2000 resulted in more efficient lignin removal and microstructure disruption of the pretreated sample, thus indicating much higher reducing sugar yields. (Cheng *et al*. 2022).

In the bioconversion process, pretreatment of lignocellulosic biomass is important to enhance the accessibility of enzyme hydrolysis, thus increased the yield of reducing sugar (Nashiruddin *et al*. 2020). Therefore, surfactant pretreatment can have a significant impact on the glucose yield in the enzymatic hydrolysis of lignocellulosic biomass. However, the effectiveness can vary depending on the specific type of surfactant used, its concentration, and the specific conditions of the pretreatment and hydrolysis processes.

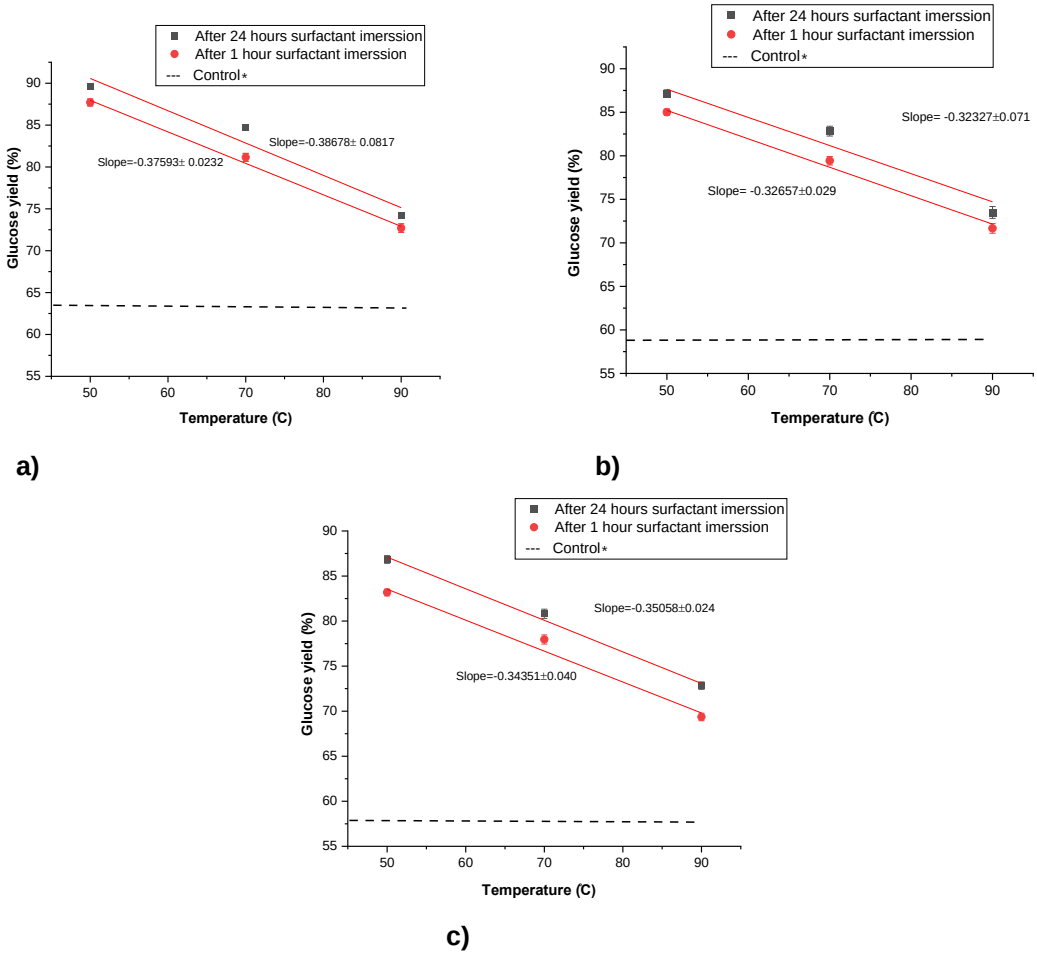



**Fig. 4.** Comparison of the effect of surfactant immersion time on glucose yield for the samples were subjected to surfactant for 1 h and 24 h. The alkaline concentrations of $Na_2CO_3$/Ca $(OH)_2$ were a) 0.5/0.5M, b) 0.4/0.4M, and c) 0.5/0.25M (Control*: the samples without surfactant and without sonication pretreatment).

As the results in Fig. 4 showed, in comparison to the effectiveness of sonication temperature and surfactant immersion time on glucose yield, the effect of temperature was more significant. Comparing to our previous work (Zhand *et al*. 2020) in which the optimum glucose yield was 64.2% for the samples pretreated with 0.6/0.5 M $Na_2CO_3$/$Ca(OH)_2$, and the current work in which the highest amount of glucose yield was (89.6%), surfactant-assisted sonication increased glucose yield increased to 25%.

Figure 4a also illustrated that when samples were subjected to 0.5/0.5M of $Na_2CO_3$/Ca $(OH)_2$ pretreatment, the highest amount of glucose yield was 89.6% and by increasing the surfactant immersion time from 1 h to 24 h the amount of glucose yield will increase 1.4~3.5% which is remarkable. As the results showed, by increasing the sonication temperature the amount of glucose yield decreased to 74.1% which was explained above.

Figure 4 (b, c) showed different concentrations of alkaline pretreatment and the amount of increase in glucose yield after 24 h of surfactant immersion was up to 3.6%. Comparing the results of glucose yield after alkaline pretreatment (Fig. 1) and after surfactant-assisted sonication (Fig. 4), it was determined that surfactant associated sonication could increase the glucose yield 26.2% more than when the samples were pretreated only by alkaline 0.5/0.5 M of $Na_2CO_3$/Ca $(OH)_2$.

As shown in Fig. 4a, the amount of glucose yield after increasing the sonication temperature to 90 °C decreased up to 15.4% for the samples immersed in surfactant for 24 h, whereas this amount reached to 13.6% for samples in Fig. 4b and 14% for samples in Fig. 4c. As has been demonstrated, increasing the sonication temperature more than 50 °C was not desirable. The optimum sonication temperature for alkali-pretreated *Eucalyptus* biomass was 50 °C. Also, immersion of surfactant in slurry before the enzymatic hydrolysis can increase glucose yield. The optimum glucose yield in current work was 89.6% at 50 °C sonication temperature and 24 h surfactant immersion for the samples pretreated with 0.5/0.5 M of $Na_2CO_3$/Ca $(OH)_2$.

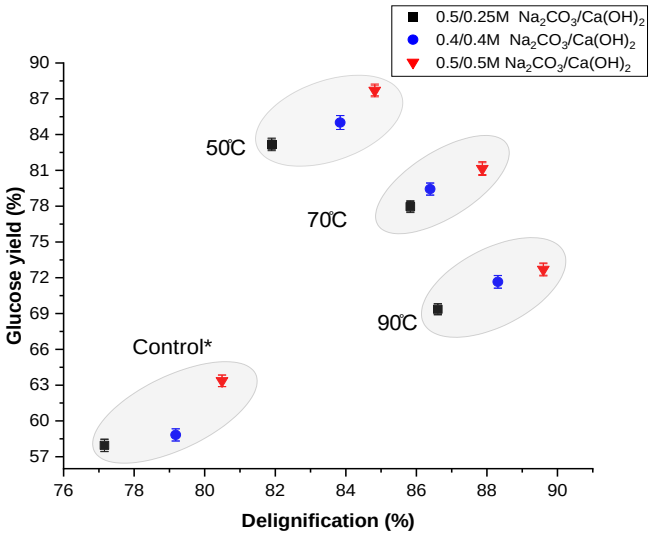



**Fig. 5.** Comparison of the delignification and glucose yield after enzymatic hydrolysis between the samples pretreated at different temperatures with 3% surfactant for 1 h immersed and 1 h sonication (Control*: the samples without surfactant and without sonication pretreatment)

As shown in Fig. 5, the glucose yield for the samples subjected to 0.5/0.5M of $Na_2CO_3$/$Ca(OH)_2$ pretreatment during immersion in surfactant for 1 hour and sonication at 50°C for 1 hour was 87.7%. Comparing the results of delignification and glucose yield

after alkaline pretreatment and after surfactant-assisted sonication (Fig. 5), it was determined that 1 h surfactant immersion associated with sonication at 50 °C could increase the glucose yield 24.3% more than the samples that were pretreated only by 0.5/0. 5M of $Na_2CO_3$/Ca $(OH)_2$. The results also showed that there was a 13.3% to 15% decrease in glucose yield by increasing the sonication temperature from 50 to 90 °C. The rise in temperature causing a rise in delignification is attributed to the impact of cavitation throughout the treatment duration. The implosion of cavitating bubbles results in the generation of significant energy and turbulence, facilitating the breakdown of lignin and promoting the efficacy of alkali in lignin removal. Consequently, this led to an augmentation in the extent of delignification (Patil and Rathod 2020). Kininge and Gotate (2022) evaluated the effect of sonication on alkaline delignification of sugarcane bagasse. The rate of delignification increased by increasing the sonication temperature from 50 to 70 °C by up to 66.4% (Kininge and Gogate 2022). Compared to the results of the experiment done by other scientists, the highest amount of glucose yield will be reached with ultrasound assisted alkaline pretreatment at an optimum temperature of 50 °C (Li *et al*. 2016; He *et al*. 2017).

When analyzing Fig. 5, it might be asked why the amount of glucose yield after pretreatment by 0.5/0.25M of $Na_2CO_3$/Ca $(OH)_2$ at 50 °C sonication temperature was 83.1% and the ones pretreated by 0.5/0.5M of $Na_2CO_3$/Ca $(OH)_2$ at 70 °C sonication temperature was 81.1%, meaning that the differences was just 2%. Also, the delignification differences between these two samples were 5.9%. This could be explained based on the fact that to reach the higher amount of glucose yield, it is not reasonable to assume higher temperature (70 °C) and higher alkaline concentration (0.5/0.5), while it's possible to reach to this point with less energy consumption.

The data in Fig. 5 was for the samples immersed in surfactant for 1 h, whereas the results for the sample which were immersed for 24 h in surfactant prior to enzymatic hydrolysis are shown and discussed in Figs. 2 and 4. The results in Fig. 5 showed that by increasing the sonication temperature, the amount of glucose after enzymatic hydrolysis decreased, which means that the effectiveness of surfactant-assisted sonication depends on sonication temperature. Elevated temperature treatment can result in the breakdown of hemicelluloses. Consequently, there is a notable reduction in the quantity of available hydroxyl groups on the fiber surface, accompanied by a decline in surface polarity, an augmentation in surface roughness, and an increase in crystallinity (Akgül *et al*. 2007). Lignin removal in sugarcane bagasse ultrasonic pretreatment led to as much as a two-fold increase of the enzymatic hydrolysis (Velmurugan and Muthukumar 2012b). A reduction in lignin content through ultrasonic pretreatment of corn stover has been observed to enhance hydrolysis yields (Zhang *et al*. 2008). Ultrasonic treatment resulted in delignification across diverse lignocellulosic materials using various solvents (Bussemaker and Zhang 2013; Sasmal *et al*. 2012; Velmurugan and Muthukumar 2012b). Overall, the delignification that has been reported ranges from an increase in lignin content by 8.9% in a soda solution (García *et al*. 2011) to a decrease in lignin by 90.6% in 2% NaOH (Velmurugan and Muthukumar 2012a). A study by Chang *et al*. examined the impact of ultrasound-ionic liquid pretreatment, supplemented with sodium dodecyl sulfate (SDS), on the breakdown of water hyacinth. They found that this method boosted the production of reducing sugars, cellulose conversion, and delignification by 72.2%, 58.7%, and 21.0%, respectively (Chang *et al*. 2017).

The generation of heat and pressure during cavitation induces a rise in the sonication fluid's temperature. This elevated temperature, in turn, amplifies the vapor

pressure of the liquid, facilitating a more effortless occurrence of cavitation (Morton *et al*. 2021). The findings of Wu *et al*. (2017) involving ultrasound-assisted alkaline pretreatment (NaOH 1% w/v) to improve enzymatic hydrolysis of rice straw, utilizing heat energy from ultrasonication, revealed a marginal elevation in the crystallinity index of rice straw. This increase can be attributed to the removal of lignin and hemicellulose during alkaline pretreatment with NaOH. Employing heat energy dissipated from ultrasonication, ultrasound-assisted alkaline pretreatment demonstrated superior efficacy in reducing the crystallinity of rice straw. Furthermore, the concentration of reducing sugar achieved an impressive 3.5-fold increase compared to control samples, reaching 60.8% (Wu *et al*. 2017). Garcia *et al*. (2012) examined the impact of ultrasound treatment on the physicochemical characteristics of alkaline lignin. The findings revealed that ultrasound cleaved lignin–carbohydrate linkages, enhancing the removal of hemicellulose impurities from the alkaline sample (up to 32% reduction in initial carbohydrate content). Additionally, the treatment influenced the insoluble lignin content, leading to insoluble lignin content (19% of Klason lignin solubilization) (García *et al*. 2012).

Eventually, there is a notable relationship between alkaline content and sonication temperature on delignification and glucose yield. Increased alkaline content and sonication temperature correlated with higher delignification, and increased sonication temperature caused lower glucose yield, as explained above. In summary, there should be balanced approach to optimize the alkaline content and sonication temperature to reach reasonable glucose yield and delignification results. In the present work, the optimum condition was 0.5/0. 5M of $Na_2CO_3$/Ca $(OH)_2$ pretreatment and 24 h surfactant immersion time and sonication at 50 °C.

Enzymatic hydrolysis, a crucial process in the conversion of biomass into biofuels and biochemicals, involves the breakdown of complex carbohydrates into simpler sugars by enzymes. One of the primary products of this process is glucose, a monosaccharide that serves as a vital energy source for many organisms and a critical raw material in various industrial applications (Cai *et al*. 2019). Recent studies have indicated a positive correlation between the duration of enzymatic hydrolysis and the yield of glucose. As the hydrolysis process progressed, the enzymes continued to act on the biomass, breaking down more complex carbohydrates and increasing the overall glucose yield. This trend suggested that by optimizing the duration of enzymatic hydrolysis, it might be possible to enhance glucose production, thereby improving the efficiency and economic viability of biofuel and biochemical production processes (Cai *et al*. 2019; Oliveira *et al*. 2021).

As shown in Fig. 6, there was a direct relationship between enzymatic hydrolysis glucose yield and time. Figure 6a shows that for the samples pretreated with alkaline concentration 0.5/0.5M of $Na_2CO_3$/Ca $(OH)_2$ and different surfactant immersion times and different sonication temperatures with the prolongation of enzymatic hydrolysis time, the glucose yield at first increased rapidly, and then there was little change. The initial glucose yield after enzymatic hydrolysis of 0.5/0.5M were similar to those at different surfactant immersion time with different sonication temperature. After 72 h of enzymatic hydrolysis, the effect of surfactant immersion time and sonication temperature was revealed. The highest glucose yield was 89.6% for the samples immersed in Tween-40 for 24 h and sonicated at 50 °C. The present results were similar to the results of Zhao *et al*. (2023), which were focused on improving reducing sugar of wheat straw by enzymatic hydrolysis. They removed 80.6% of lignin and 78.5% hemicellulose, while they maintained 87.4% cellulose at 90 °C for 5 h, resulting in 81.6% reducing sugar produced during hydrolysis for 72 h (Zhao *et al*. 2023).

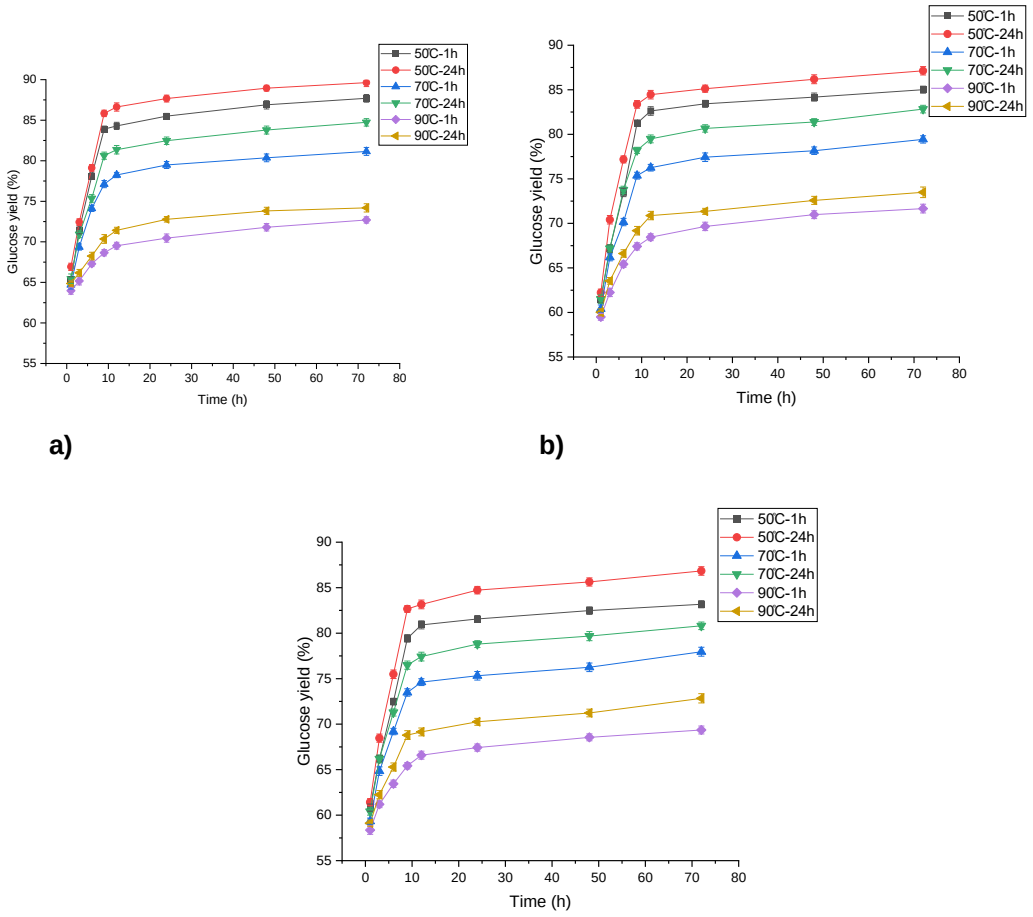



**Fig. 6.** Effect of enzymatic hydrolysis time on the rate of glucose yield for the samples which were immersed in surfactant for 1 h and 24 h. a) Samples were pretreated with alkaline concentration of $Na_2CO_3$/Ca $(OH)_2$ of 0.5/0. 5M, b) 0.4/0.4M, c) 0.5/0.25M.

As the results showed after alkaline and surfactant-assisted sonication pretreatment, the amount of glucose content reached 37.02 g out of 51.7 g of glucose in *Eucalyptus*, which means that 71.6% of glucose will be achieved after this combination of pretreatments. After enzymatic hydrolysis, the amount of glucose conversion reached approximately 90%, which demonstrates that this kind of pretreatment specifically effects cellulose degradation. Implementation of this specific pretreatment technique (surfactant-assisted sonication of alkaline pretreatment) results in a reduction of the total glucose content. However, it simultaneously enhances the efficiency of glucose extraction, thereby leading to an increase in the overall yield of glucose.

Xu *et al*. (2121) did an experiment on sugar recovery from enzymatic hydrolysis of *Miscanthus sinensis* by surfactant mediated alkaline pretreatment. They found that a surfactant-mediated alkaline pretreatment process resulted in significantly more hemicellulose removal, more efficient delignification, higher cellulose content (55% *vs*. 51%), and stronger morphological disruption of the pretreated stalks, making them more accessible to cellulases and/or microorganisms and produced a significantly higher sugar yield (Xu *et al*. 2021). The results of the study showed that surfactant pretreatment could decrease the amount of glucose content but increase the glucose yield in the enzymatic hydrolysis of lignocellulosic biomass.

Also, the application of sonication pretreatment can lead to a decrease in the total glucose content in the biomass. This is because the ultrasound waves can cause the disruption of cellulose particles, leading to a significant decrease in the particle size of cellulose. This disruption can also result in the formation of large pores and partial fibrillation (SriBala *et al*. 2016).

Despite the decrease in total glucose content, the glucose yields increased. This is because the sonication pretreatment enhanced the accessibility of the cellulose to the enzymes, and thereby improved the efficiency of the enzymatic hydrolysis process. More specifically, the pretreatment can lead to a great disruption of cellulose particles along with the formation of large pores and partial fibrillation. These changes can enhance the accessibility of the enzymes to the cellulose, leading to an increase in the glucose yield (SriBala *et al*. 2016).

*Surface morphology through FE-SEM*

The surface morphology of the control and alkaline pretreated with surfactant-assisted sonication samples was determined using FE-SEM analysis. FE-SEM analysis images of *Eucalyptus pellita* exposed to various pretreatments are illustrated in Fig. 7. It is obvious from Fig. 7 (a, a') that the unpretreated samples showed a compact nature with strong binding of the cell wall. After alkaline pretreatment ($Na_2CO_3$/Ca $(OH)_2$) and surfactant-assisted sonication, the structure swelled with weak binding of the cell wall, so the wood structures swelled. Loosening of the cell wall also led to increased surface area as shown in Fig. 7 (c, e, and g).

Alkaline pretreatment is known to induce changes in the surface morphology of wood fibers. Studies by Lima *et al*. (2013) and Hashim *et al*. (2017) showed that alkaline pretreatment affected the surface structure and physical properties of fibers, leading to improved bending strength, water resistance, and thermal stability (Lima *et al*. 2013; Hashim *et al*. 2017). Figure 7(a, a') shows the surface of alkaline pretreated samples covered with residues due to the alkaline pretreatment process. The total amount of residues on the sample surfaces decreased after surfactant-assisted sonication pretreatment (Fig. 7b through g).

The surfactants' hydrophilic and hydrophobic attributes reduce the surface tension between liquid phases during pretreatment, facilitating the elimination of hydrophobic compounds like lignin (Kataria *et al*. 2018). The findings suggest that the introduction of surfactants amplifies the alkali-induced disruption of cell walls, leading to increased formation of cracks, fragments, and removal of lignin. As a result, more reactive sites on the biomass surface are generated. These alterations play a crucial role in diminishing the non-productive absorption of cellulases, enhancing the accessibility of samples to cellulases, and thereby boosting the subsequent enzymatic hydrolysis (Wang *et al*. 2020b).

As illustrated in Fig. 7b and c, the effect of surfactant immersion time on the surface morphology of the fibers indicated that by increasing the surfactant immersion time, the partial fibrillation of fibers will increase. Comparing Fig. 7 c, e, and g, it becomes clear that increasing the sonication temperature did not have a significant effect on morphological disruption of the fibers, but the effect of surfactant immersion time was more significant. For the sonication associated with alkali pretreatment, similar images were reported by Kininge and Gogate (2022). The investigation conducted by Gupta and Tuohy (2013) on alkaline pretreatment assisted by sonication revealed the disintegration of the cell matrix due to cavitation effects. This process resulted in a reduction in particle size and an increase in surface area (Gupta and Tuohy 2013).

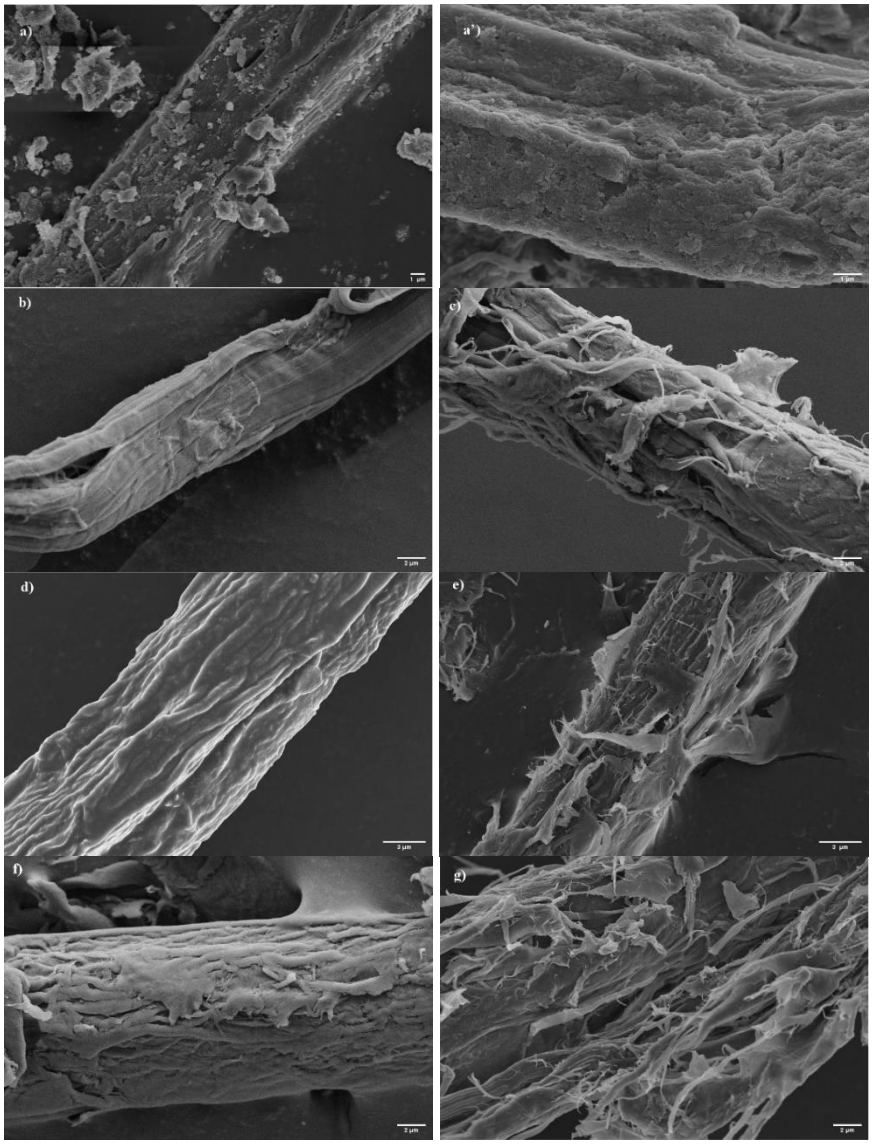



**Fig. 7.** Scanning Electron Microscope images of the samples a), a´) control samples after alkaline pretreatment in 0.5:0.5 molar ratio of $Na_2CO_3$:$Ca(OH)_2$ and b) 1 h immersion in surfactant and sonication in 50 °C, c) after 24 h immersion in surfactant and sonication in 50 °C, d) after 1 h immersion in surfactant and sonication in 70°C, e) after 24 h immersion in surfactant and sonication in 70 °C, f) after 1 h immersion in surfactant and sonication in 90 °C, g) after 24 h immersion in surfactant and sonication in 90 °C

## CONCLUSIONS

1. The effects of different pretreatments to reach the optimum glucose yield were examined. The variables were alkaline concentration, surfactant immersion time, and sonication temperature. Applying alkaline pretreatment alone has its own obstacles and cannot increase glucose yield as desired. This is the case even though applying strong alkaline has its own limitations. So, to overcome the limitations of applying only alkaline pretreatment, ultrasound assisted surfactant pretreatment were used. This work focused on explaining the role of surfactants for lignocellulose substrates and finding

the optimum temperature range which surfactant-assisted sonication can work well, which resulted in removing up to 90% of lignin with 89.6% glucose yield.

2. Immersing the surfactant in the slurry for 24 h enhanced substrate disruption, making cellulose more accessible to enzymatic attack. Additionally, the surfactant played a crucial role in preventing unproductive binding of cellulases to lignin. The primary mechanism for surfactant effects in substrates containing lignin lies in the surfactant's influence on enzyme interaction with lignin surfaces. Surfactant adsorption to lignin effectively inhibit unproductive enzyme binding to lignin.
3. Furthermore, the study explored the impact of sonication temperature, aiming to identify the optimal temperature for effective ultrasonic effects. The investigation revealed that 50 °C was the optimum temperature. Higher temperatures, while enhancing delignification, adversely affected glucose yield. Elevated temperatures, particularly at 90 °C, reduced gas solubility in the liquid, negatively impacted cavitation by causing slower collapsing of cavitation bubbles during the compressive phase of sonication.
4. In this work it was determined that applying alkaline pretreatment and surfactant associated sonication led to a decrease in the amount of glucose, but the final glucose yield after enzymatic hydrolysis was increased to 89.6%. In future research, there would be possibilities to evaluate different alkaline pretreatments in surfactant-assisted sonication pretreatment to compare the differences. Also, further work is needed to analyze the effect of oxidizing agent on delignification and glucose yield other than surfactant and sonication on softwoods to investigate the effect of this kind of pretreatment, on the degree of crystallinity of cellulose.

## ACKNOWLEDGMENTS

The authors acknowledge the financial support from the 2023 Cultural Heritage Smart Preservation and Utilization R&D Program of the Cultural Heritage Administration, National Research Institute of Cultural Heritage (Project name: Development of alternative technology for fumigation using radiation for emergency preservation in response to disasters in cultural heritage, Project Number: 2023A01D07-001-2, Contribution Rate: 100%).

## REFERENCES CITED

Agbor, V. B., Cicek, N., Sparling, R., Berlin, A., and Levin, D. B. (2011). "Biomass pretreatment: Fundamentals toward application," *Biotechnology Advances* 29(6), 675-685. DOI: 10.1016/j.biotechadv.2011.05.005

Ahenkorah, I., Rahman, M. M., Karim, M. R., and Beecham, S. (2021). "Enzyme induced calcium carbonate precipitation and its engineering application: A systematic review and meta-analysis," *Construction and Building Materials* 308, article 125000. DOI: 10.1016/j.conbuildmat.2021.125000

Akgül, M., Gümüşkaya, E., and Korkut, S. (2007). "Crystalline structure of heat-treated Scots pine [*Pinus sylvestris* L.] and Uludağ fir [*Abies nordmanniana* (Stev.) subsp. *bornmuelleriana* (Mattf.)] wood," *Wood Science and Technology* 41(3), 281-289.

DOI: 10.1007/s00226-006-0110-9

Balat, M., Balat, H., and Öz, C. (2008). "Progress in bioethanol processing," *Progress in Energy and Combustion Science* 34(5), 551-573. DOI: 10.1016/j.pecs.2007.11.001

Broda, M., Yelle, D. J., and Serwańska, K. (2022). "Bioethanol production from lignocellulosic biomass—challenges and solutions," *Molecules* 27(24), 8717. DOI: 10.3390/molecules27248717

Brown, R. C. (2003). *Biorenewable Resources: Engineering New Products from Agricultural*, Iowa State Press, Ames, IA, USA.

Bussemaker, M. J., and Zhang, D. (2013). "Effect of ultrasound on lignocellulosic biomass as a pretreatment for biorefinery and biofuel applications," *Industrial and Engineering Chemistry Research* 52(10), 3563-3580. DOI: 10.1021/ie3022785

Cai, C., Bao, Y., Zhan, X., Lin, X., Lou, H., Pang, Y., Qian, Y., and Qiu, X. (2019). "Recovering cellulase and increasing glucose yield during lignocellulosic hydrolysis using lignin-MPEG with a sensitive pH response," *Green Chemistry* 21(5), 1141-1151. DOI: 10.1039/C8GC04059A

Chang, K.-L., Han, Y.-J., Wang, X.-Q., Chen, X.-M., Leu, S.-Y., Liu, J.-y., Peng, Y.-P., Liao, Y.-L., and Potprommanee, L. (2017). "The effect of surfactant-assisted ultrasound-ionic liquid pretreatment on the structure and fermentable sugar production of a water hyacinth," *Bioresource Technology* 237, 27-30. DOI: 10.1016/j.biortech.2017.02.044

Cheng, X., Luo, Y., Gao, Y., Li, S., Xu, C., Tang, S., Yang, Y., Zhang, Z., Jiang, H., and Xu, H. (2022). "Surfactant-assisted alkaline pretreatment and enzymatic hydrolysis of Miscanthus sinensis for enhancing sugar recovery with a reduced enzyme loading. *Frontiers in Bioengineering and Biotechnology* 10, article 918126. DOI: 10.3389/fbioe.2022.918126

Cherubini, F., and Ulgiati, S. (2010). "Crop residues as raw materials for biorefinery systems–A LCA case study," *Applied Energy* 87(1), 47-57. DOI: 10.1016/j.apenergy.2009.08.024

Chisti, Y. (2003). "Sonobioreactors: Using ultrasound for enhanced microbial productivity," *Trends in Biotechnology* 21(2), 89-93. DOI: 10.1016/S0167-7799(02)00033-1

Coppola, G., Gaudio, M. T., Lopresto, C. G., Calabro, V., Curcio, S., and Chakraborty, S. (2021). "Bioplastic from renewable biomass: A facile solution for a greener environment," *Earth Systems and Environment* 5, 231-251. DOI: 10.1007/s41748-021-00208-7

de Carvalho, D. M., de Queiroz, J. H., and Colodette, J. L. (2016). "Assessment of alkaline pretreatment for the production of bioethanol from eucalyptus, sugarcane bagasse and sugarcane straw," *Industrial Crops and Products* 94, 932-941. DOI: 10.1016/j.indcrop.2016.09.069

Du, B., Chen, C., Sun, Y., Yang, M., Yu, M., Liu, B., Wang, X., and Zhou, J. (2020). "Efficient and controllable ultrasound-assisted depolymerization of organosolv lignin catalyzed to liquid fuels by MCM-41 supported phosphotungstic acid," *RSC Advances* 10(52), 31479-31494. DOI: 10.1039/D0RA05069E

Elalami, D., Fertahi, S., Aouine, M., Benali, W., Ibnyasser, A., Lyamlouli, K., and Barakat, A. (2022). "Comparison of pretreatment effects on sugar release, energy efficiency and the reuse of effluents," *Industrial Crops and Products* 189, article 115769. DOI: 10.1016/j.indcrop.2022.115769

Eriksson, T., Börjesson, J., and Tjerneld, F. (2002). "Mechanism of surfactant effect in

enzymatic hydrolysis of lignocellulose," *Enzyme and Microbial Technology* 31(3), 353-364. DOI: 10.1016/S0141-0229(02)00134-5

Fadwati, A. D., Hidayati, F., and Na'iem, M. (2023). "Evaluation of genetic parameters of growth characteristics and basic density of *Eucalyptus pellita* clones Planted at two different sites in East Kalimantan, Indonesia," *Journal of the Korean Wood Science and Technology* 51(3), 222-237. DOI: 10.5658/WOOD.2023.51.3.222

Galbe, M., and Zacchi, G. (2012). "Pretreatment: The key to efficient utilization of lignocellulosic materials," *Biomass and Bioenergy* 46, 70-78. DOI: 10.1016/j.biombioe.2012.03.026

García, A., Alriols, M. G., Llano-Ponte, R., and Labidi, J. (2011). "Ultrasound-assisted fractionation of the lignocellulosic material," *Bioresource Technology* 102(10), 6326-6330. DOI: 10.1016/j.biortech.2011.02.045

García, A., Erdocia, X., Alriols, M.G., and Labidi, J. (2012). "Effect of ultrasound treatment on the physicochemical properties of alkaline lignin," *Chemical Engineering and Processing: Process Intensification* 62, 150-158. DOI: 10.1016/j.cep.2012.07.011

Gaudino, E. C., Grillo, G., Tabasso, S., Stevanato, L., Cravotto, G., Marjamaa, K., Pihlajaniemi, V., Koivula, A., Aro, N., and Uusitalo, J. (2022). "Optimization of ultrasound pretreatment and enzymatic hydrolysis of wheat straw: From lab to semi-industrial scale," *Journal of Cleaner Production* 380, article 134897. DOI: 10.1016/j.jclepro.2022.134897

Ghavi, F. P., Raouf, F., and Koohi, A. D. (2021). "The effect of alkaline pretreatment on surfactant-modified clinoptilolite for diclofenac adsorption: Isotherm, kinetic, and thermodynamic studies," *Journal of Water and Health* 19(1), 47-66. DOI: 10.2166/wh.2020.057

Guigou, M., Cabrera, M. N., Vique, M., Bariani, M., Guarino, J., Ferrari, M. D., and Lareo, C. (2019). "Combined pretreatments of eucalyptus sawdust for ethanol production within a biorefinery approach," *Biomass Conversion and Biorefinery* 9, 293-304. DOI: 10.1007/s13399-018-0353-3

Guo, F., Shi, W., Sun, W., Li, X., Wang, F., Zhao, J., and Qu, Y. (2014). "Differences in the adsorption of enzymes onto lignins from diverse types of lignocellulosic biomass and the underlying mechanism," *Biotechnology for Biofuels* 7, 1-10. DOI: 10.1186/1754-6834-7-38

Gupta, V. K., and Tuohy, M. G. (2013). "Biofuel technologies. *Recent Developments. Editorial Springer*. DOI: 10.1007/978-3-642-34519-7

Hashim, M. Y., Amin, A. M., Marwah, O. M. F., Othman, M. H., Yunus, M. R. M., and Huat, N. C. (2017). "The effect of alkali treatment under various conditions on physical properties of kenaf fiber," *Journal of Physics: Conference Series*. IOP Publishing. pp. 012030. DOI: 10.1088/1742-6596/914/1/012030

He, Z., Wang, Z., Zhao, Z., Yi, S., Mu, J., and Wang, X. (2017). "Influence of ultrasound pretreatment on wood physiochemical structure," *Ultrasonics Sonochemistry* 34, 136-141. DOI: 10.1016/j.ultsonch.2016.05.035

Hendriks, A., and Zeeman, G. (2009). "Pretreatments to enhance the digestibility of lignocellulosic biomass," *Bioresource Technology* 100(1), 10-18. DOI: 10.1016/j.biortech.2008.05.027

Kaar, W. E., and Holtzapple, M. T. (1998). "Benefits from Tween during enzymic hydrolysis of corn stover," *Biotechnology and Bioengineering* 59(4), 419-427. DOI: 10.1002/(SICI)1097-0290(19980820)59:4<419::AID-BIT4>3.0.CO;2-J

Kaar, W. E., and Holtzapple, M. T. (2000). "Using lime pretreatment to facilitate the enzymic hydrolysis of corn stover," *Biomass and Bioenergy* 18(3), 189-199. DOI: 10.1016/S0961-9534(99)00091-4

Kardos, N., and Luche, J.-L. (2001). "Sonochemistry of carbohydrate compounds," *Carbohydrate Research* 332(2), 115-131. DOI: 10.1016/S0008-6215(01)00081-7

Karimi, K., and Taherzadeh, M. J. (2016). "A critical review on analysis in pretreatment of lignocelluloses: Degree of polymerization, adsorption/desorption, and accessibility," *Bioresource Technology* 203, 348-356. DOI: 10.1016/j.biortech.2015.12.035

Kataria, R., Woods, T., Casey, W., Cerrone, F., Davis, R., O'Connor, K., Ruhal, R., and Babu, R. (2018). "Surfactant-mediated hydrothermal pretreatment of Ryegrass followed by enzymatic saccharification for polyhydroxyalkanoate production," *Industrial Crops and Products* 111, 625-632. DOI: 10.1016/j.indcrop.2017.11.029

Kaya, F., Heitmann, J., and Joyce, T. W. (1995). "Influence of surfactants on the enzymatic-hydrolysis of xylan and cellulose," *Tappi Journal* 78(10).

Kim, T. H., Frank, T., and Kevin B. H. (2008). "Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment," *Bioresource technology* 99, no. 13, 5694-5702. DOI: 10.1016/j.biortech.2007.10.055

Kim, J. S., Lee, Y., and Kim, T. H. (2016). "A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass," *Bioresource Technology* 199, 42-48. DOI: 10.1016/j.biortech.2015.08.085

Kim, M., Lee, S., Ryu, D. D., and Reese, E. (1982). "Surface deactivation of cellulase and its prevention," *Enzyme and Microbial Technology* 4(2), 99-103. DOI: 10.1016/0141-0229(82)90090-4

Kim, T. H., and Kim, T. H. (2014). "Overview of technical barriers and implementation of cellulosic ethanol in the US," *Energy* 66, 13-19. DOI: 10.1016/j.energy.2013.08.008

Kim, T. H., and Lee, Y. (2006). "Fractionation of corn stover by hot-water and aqueous ammonia treatment," *Bioresource Technology* 97(2), 224-232. DOI: 10.1016/j.biortech.2005.02.040

Kim, Y., Ximenes, E., Mosier, N. S., and Ladisch, M. R. (2011). "Soluble inhibitors/ deactivators of cellulase enzymes from lignocellulosic biomass," *Enzyme and Microbial Technology* 48(4-5), 408-415. DOI: 10.1016/j.enzmictec.2011.01.007

Kininge, M. M., and Gogate, P. R. (2022). "Intensification of alkaline delignification of sugarcane bagasse using ultrasound assisted approach," *Ultrasonics Sonochemistry* 82, article 105870. DOI: 10.1016/j.ultsonch.2021.105870

Krishania, M., Vijay, V., and Chandra, R. (2013). "Methane fermentation and kinetics of wheat straw pretreated substrates co-digested with cattle manure in batch assay," *Energy* 57, 359-367. DOI: 10.1016/j.energy.2013.05.028

Kristensen, J. B., Börjesson, J., Bruun, M. H., Tjerneld, F., and Jørgensen, H. (2007). "Use of surface active additives in enzymatic hydrolysis of wheat straw lignocellulose," *Enzyme and Microbial Technology* 40(4), 888-895. DOI: 10.1016/j.enzmictec.2006.07.014

Kumar, A. K., and Sharma, S. (2017). "Recent updates on different methods of pretreatment of lignocellulosic feedstocks: A review," *Bioresources and Bioprocessing* 4(1), 1-19. DOI: 10.1186/s40643-017-0137-9

Lee, S., Akeprathumchai, S., Bundidamorn, D., Salaipeth, L., Poomputsa, K., Ratanakhanokchai, K., Chang, K.-L., and Phitsuwan, P. (2021). "Interplays of

enzyme, substrate, and surfactant on hydrolysis of native lignocellulosic biomass," *Bioengineered* 12(1), 5110-5124. DOI: 10.1080/21655979.2021.1961662

Lehto, J., and Alén, R. (2013). "Alkaline pre-treatment of hardwood chips prior to delignification," *Journal of Wood Chemistry and Technology* 33(2), 77-91. DOI: 10.1080/02773813.2012.748077

Li, S., Yang, X., Zhang, Y., Ma, H., Liang, Q., Qu, W., He, R., Zhou, C., and Mahunu, G. K. (2016). "Effects of ultrasound and ultrasound assisted alkaline pretreatments on the enzymolysis and structural characteristics of rice protein," *Ultrasonics Sonochemistry* 31, 20-28. DOI: 10.1016/j.ultsonch.2015.11.019

Li, X., Shi, Y., Kong, W., Wei, J., Song, W., and Wang, S. (2022). "Improving enzymatic hydrolysis of lignocellulosic biomass by bio-coordinated physicochemical pretreatment—A review," *Energy Reports* 8, 696-709. DOI: 10.1016/j.egyr.2021.12.015

Lima, M. A., Lavorente, G. B., da Silva, H. K., Bragatto, J., Rezende, C. A., Bernardinelli, O. D., Deazevedo, E. R., Gomez, L. D., McQueen-Mason, S. J., and Labate, C. A. (2013). "Effects of pretreatment on morphology, chemical composition and enzymatic digestibility of eucalyptus bark: a potentially valuable source of fermentable sugars for biofuel production–part 1," *Biotechnology for Biofuels* 6, 1-17. DOI: 10.1186/1754-6834-6-75

Luo, J., Fang, Z., and Smith Jr, R. L. (2014). "Ultrasound-enhanced conversion of biomass to biofuels," *Progress in Energy and Combustion Science* 41, 56-93. DOI: 10.1016/j.pecs.2013.11.001

Malmsten, M., and Van Alstine, J. M. (1996). "Adsorption of poly (ethylene glycol) amphiphiles to form coatings which inhibit protein adsorption," *Journal of Colloid and Interface Science* 177(2), 502-512. DOI: 10.1006/jcis.1996.0064

Martinez-Hernandez, E., Sadhukhan, J., and Campbell, G. M. (2013). "Integration of bioethanol as an in-process material in biorefineries using mass pinch analysis," *Applied Energy* 104, 517-526. DOI: 10.1016/j.apenergy.2012.11.054

Melro, E., Valente, A. J., Antunes, F. E., Romano, A., and Medronho, B. (2021). "Enhancing lignin dissolution and extraction: The effect of surfactants," *Polymers* 13(5), 714. DOI: 10.3390/polym13050714

Montalbo-Lomboy, M., Johnson, L., Khanal, S.K., van Leeuwen, J.H., and Grewell, D. (2010). "Sonication of sugary-2 corn: a potential pretreatment to enhance sugar release," *Bioresource Technology* 101(1), 351-8. DOI: 10.1016/j.biortech.2009.07.075

Morton, J.A., Eskin, D.G., Grobert, N., Mi, J., Porfyrakis, K., Prentice, P., and Tzanakis, I. (2021). "Effect of temperature and acoustic pressure during ultrasound liquid-phase processing of graphite in water," *JOM* 73, 3745-3752. DOI: 10.1007/s11837-021-04910-9

Nakagame, S., Chandra, R.P., Kadla, J.F., and Saddler, J.N. (2011). "Enhancing the enzymatic hydrolysis of lignocellulosic biomass by increasing the carboxylic acid content of the associated lignin," *Biotechnology and Bioengineering* 108(3), 538-548. DOI: 10.1002/bit.22981

Nashiruddin, N. I., Manas, N. H. A., Rahman, R. A., Azelee, N. I. W., Dailin, D. J., and Shaarani, S. M. (2020). "Pretreatment and enzymatic hydrolysis of lignocellulosic biomass for reducing sugar production," *Valorisation of Agro-industrial Residues–Volume II: Non-Biological Approaches* 2020, 1-27. DOI: 10.1007/978-3-030-39208-6_1

Olguin-Maciel, E., Jiménez-Villarreal, I. A., Toledano-Thompson, T., Alzate-Gaviria, L., and Tapia-Tussell, R. (2022). "Effect of ultrasound pretreatment combined with enzymatic hydrolysis of non-conventional starch on sugar yields to bioethanol conversion," *Biomass Conversion and Biorefinery* 2022, 1-9. DOI: 10.1007/s13399-022-03217-2

Oliveira, T., Interlandi, M., Hanlon, K., Torres-Mayanga, P., Silvello, M., Timko, M., Rostagno, M., Goldbeck, R., and Forster-Carneiro, T. (2021). "Integration of subcritical water and enzymatic hydrolysis to obtain fermentable sugars and second-generation ethanol from sugarcane straw," *BioEnergy Research* 2021, 1-12. DOI: 10.1007/s12155-021-10274-4

Onu Olughu, O., Tabil, L. G., and Dumonceaux, T. (2021). "Ultrasonic delignification and microstructural characterization of switchgrass," *Energies* 14(2), 263. DOI: 10.3390/en14020263

Ooshima, H., Sakata, M., and Harano, Y. (1986). "Enhancement of enzymatic hydrolysis of cellulose by surfactant. *Biotechnology and bioengineering* 28(11), 1727-1734. DOI: 10.1002/bit.260281117

Oriez, V., Peydecastaing, J., and Pontalier, P.-Y. (2020). "Lignocellulosic biomass mild alkaline fractionation and resulting extract purification processes: Conditions, yields, and purities," *Clean Technologies* 2(1), 91-115. DOI: 10.3390/cleantechnol2010007

Pareek, N., Gillgren, T., and Jönsson, L. J. (2013). "Adsorption of proteins involved in hydrolysis of lignocellulose on lignins and hemicelluloses," *Bioresource Technology* 148, 70-77. DOI: 10.1016/j.biortech.2013.08.121

Park, Y. C., and Kim, J. S. (2012). "Comparison of various alkaline pretreatment methods of lignocellulosic biomass," *Energy* 47(1), 31-35. DOI: 10.1016/j.energy.2012.08.010

Parnthong, J., Kungsanant, S., and Chavadej, S. (2018). "The influence of nonionic surfactant adsorption on enzymatic hydrolysis of oil palm fruit bunch," *Applied Biochemistry and Biotechnology* 186, 895-908. DOI: 10.1007/s12010-018-2783-6

Patil, S. S., and Rathod, V. K. (2020). "Synergistic effect of ultrasound and three phase partitioning for the extraction of curcuminoids from *Curcuma longa* and its bioactivity profile," *Process Biochemistry* 93, 85-93. DOI: 10.1016/j.procbio.2020.02.031

Prabhu, A. V., Gogate, P. R., and Pandit, A. B. (2004). "Optimization of multiple-frequency sonochemical reactors," *Chemical Engineering Science* 59(22-23), 4991-4998. DOI: 10.1016/j.ces.2004.09.033

Prabhudesai, V. S., Saravanakumar, K., Gurrala, L., and Vinu, R. (2023). "Ultrasound-assisted low-temperature catalytic lignin-first depolymerization of pine wood biomass to selectively produce propyl guaiacol," *Sustainable Energy and Fuels* 7(9), 2117-2129. DOI: 10.1039/D2SE01796B

Qing, Q., Yang, B., and Wyman, C. E. (2010). "Impact of surfactants on pretreatment of corn stover," *Bioresource Technology* 101(15), 5941-5951. DOI: 10.1016/j.biortech.2010.03.003

Rokhati, N., Kusworo, T. D., Prasetyaningrum, A., Hamada, N. A., Utomo, D. P., Riyanto, T. (2022). "Effect of surfactant HLB value on enzymatic hydrolysis of chitosan," *ChemEngineering* 6(1), article 17. DOI: 10.3390/chemengineering6010017

Sánchez-Muñoz, S., Balbino, T., Terán-Hilares, R., Mier-Alba, E., Barbosa, F., Balagurusamy, N., Santos, J., and da Silva, S. (2022). "Non-ionic surfactant formulation sequentially enhances the enzymatic hydrolysis of cellulignin from

sugarcane bagasse and the production of *Monascus ruber* biopigments," *Bioresource Technology* 362, article 127781. DOI: 10.1016/j.biortech.2022.127781

Sasmal, S., Goud, V. V., and Mohanty, K. (2012). "Ultrasound assisted lime pretreatment of lignocellulosic biomass toward bioethanol production," *Energy and Fuels* 26(6), 3777-3784. DOI: 10.1021/ef300669w

Simoni, M., Hanein, T., Woo, C. L., Nyberg, M., Tyrer, M., Provis, J. L., and Kinoshita, H. (2022). "Synthesis of Ca $(OH)_2$ and $Na_2CO_3$ through anion exchange between $CaCO_3$ and NaOH: effect of reaction temperature," *RSC Advances* 12(49), 32070-32081. DOI: 10.1039/D2RA05827H

Sindhu, R., Kuttiraja, M., Preeti, V. E., Vani, S., Sukumaran, R. K., and Binod, P. (2013). "A novel surfactant-assisted ultrasound pretreatment of sugarcane tops for improved enzymatic release of sugars," *Bioresource Technology* 135, 67-72. DOI: 10.1016/j.biortech.2012.09.050

SriBala, G., Chennuru, R., Mahapatra, S., and Vinu, R. (2016). "Effect of alkaline ultrasonic pretreatment on crystalline morphology and enzymatic hydrolysis of cellulose," *Cellulose* 23, 1725-1740. DOI: 10.1007/s10570-016-0893-2

Sun, Q., Patil, P. J., Singh, A. K., Teng, C., Zhou, M., Zhou, Y., and Fan, G. (2023). "Optimization of pretreatment and enzymatic hydrolysis coupled with ultrasonication for the production of xylooligosaccharides from corn cob," *Biomass Conversion and Biorefinery* 1-21. DOI: 10.1007/s13399-023-04145-5

Suslick, K. S. (1989). "The chemical effects of ultrasound," *Scientific American* 260(2), 80-87. DOI: 10.1038/scientificamerican0289-80

Toan, S., O'Dell, W., Russell, C.K., Zhao, S., Lai, Q., Song, H., Zhao, Y., and Fan, M. (2019). "Thermodynamics of $NaHCO_3$ decomposition during $Na_2CO_3$-based $CO_2$ capture," *Journal of Environmental Sciences* 78, 74-80. DOI: 10.1016/j.jes.2018.07.005

Velmurugan, R., and Muthukumar, K. (2012a). "Sono-assisted enzymatic saccharification of sugarcane bagasse for bioethanol production," *Biochemical Engineering Journal* 63, 1-9. DOI: 10.1016/j.bej.2012.01.001

Velmurugan, R., and Muthukumar, K. (2012b). "Ultrasound-assisted alkaline pretreatment of sugarcane bagasse for fermentable sugar production: Optimization through response surface methodology," *Bioresource Technology* 112, 293-299. DOI: 10.1016/j.biortech.2012.01.168

Wang, J.-X., Asano, S., Kudo, S., and Hayashi, J.-i. (2020a). "Deep delignification of woody biomass by repeated mild alkaline treatments with pressurized $O_2$," *ACS Omega* 5(45), 29168-29176. DOI: 10.1021/acsomega.0c03953

Wang, W., Wang, C., Zahoor, Chen, X., Yu, Q., Wang, Z., Zhuang, X., and Yuan, Z. (2020b). "Effect of a nonionic surfactant on enzymatic hydrolysis of lignocellulose based on lignocellulosic features and enzyme adsorption," *ACS Omega* 5(26), 15812-15820. DOI: 10.1021/acsomega.0c00526

Woo, C. L., Hanein, T., Nyberg, M., Martinez, J. C., Isaias, N., Mora, Q., and Kinoshita, H. (2020). "Interaction of $Na_2CO_3$ and $Ca(OH)_2$ in aqueous system for mild activation of clay materials," in: *40th Cement and Concrete Science Conference Proceedings*, University of Sheffield, Sheffield, UK

Wu, H., Dai, X., Zhou, S.-L., Gan, Y.-Y., Xiong, Z.-Y., Qin, Y.-H., Ma, J., and Yang, L., Wu, Z.-K., and Wang, T.-L. (2017). "Ultrasound-assisted alkaline pretreatment for enhancing the enzymatic hydrolysis of rice straw by using the heat energy dissipated from ultrasonication," *Bioresource Technology* 241, 70-74. DOI:

10.1016/j.biortech.2017.05.090

Wu, W., Li, P., Huang, L., Wei, Y., Li, J., Zhang, L., and Jin, Y. (2023). "The role of lignin structure on cellulase adsorption and enzymatic hydrolysis," *Biomass* 3(1), 96-107. DOI: 10.3390/biomass3010007

Xu, C.-M., Gao, Y.-F., He, S.-S., Luo, K., Yan, Q., and Cheng, X.-Y. (2021). "Improved sugar recovery from enzymatic hydrolysis of *Miscanthus sinensis* by surfactant-mediated alkaline pretreatment," *Biomass Conversion and Biorefinery*, 1-8. DOI: 10.1007/s13399-021-01491-0

Yaldagard M., Mortazavi S.-A., and Tabatabaie, F. (2008) "Application of ultrasonic waves as a priming technique for accelerating and enhancing the germination of barley seed: Optimization of method by the Taguchi approach," *Journal of the Institute of Brewing* 114(1), 14-21. DOI:10.1002/j.2050-0416.2008.tb00300.x

Yoon, S.-H., and Robyt, J. F. (2005). "Activation and stabilization of 10 starch-degrading enzymes by Triton X-100, polyethylene glycols, and polyvinyl alcohols," *Enzyme and Microbial Technology* 37(5), 556-562. DOI: 10.1016/j.enzmictec.2005.04.002

Zhand, S., Yang, B. S., Kang, K.-Y., and Jeong, M.-J. (2020). "The effects of mild alkaline pretreatment on the enzymatic hydrolysis of *Eucalyptus pellita* wood," *Journal of Korea TAPPI* 52(2), 70-77. DOI: 10.7584/JKTAPPI.2020.04.52.2.70

Zhang, H., Zhang, J., Xie, J., and Qin, Y. (2020). "Effects of NaOH-catalyzed organosolv pretreatment and surfactant on the sugar production from sugarcane bagasse," *Bioresource Technology* 312, article 123601. DOI: 10.1016/j.biortech.2020.123601

Zhang, Y., Fu, E., and Liang, J. (2008). "Effect of ultrasonic waves on the saccharification processes of lignocellulose," *Chemical Engineering and Technology: Industrial Chemistry-Plant Equipment-Process Engineering-Biotechnology* 31(10), 1510-1515. DOI: 10.1002/ceat.200700407

Zhao, X., Han, L., Ma, X., Sun, X., and Zhao, Z. (2023). "Enhanced enzymatic hydrolysis of wheat straw to improve reducing sugar yield by novel method under mild conditions," *Processes* 11(3), 898. DOI: 10.3390/pr11030898

Zhu, F., and Li, H. (2019). "Modification of quinoa flour functionality using ultrasound. *Ultrasonics Sonochemistry* 52, 305-310. DOI: 10.1016/j.ultsonch.2018.11.027

Ziaei-Rad, Z., Pazouki, M., Fooladi, J., Azin, M., Gummadi, S.N., and Allahverdi, A. (2023). "Investigation of a robust pretreatment technique based on ultrasound-assisted, cost-effective ionic liquid for enhancing saccharification and bioethanol production from wheat straw," *Scientific Reports* 13(1), article 446. DOI: 10.1038/s41598-022-27258-9

Article submitted: January 11, 2024; Peer review completed: January 27, 2024; Revised version received and accepted: February 6, 2024; Published: February 19, 2024.
DOI: 10.15376/biores.19.2.2244-2271