

Article

Synergistic Effects of Torrefaction and Alkaline Pretreatment on Sugar and Bioethanol Production from Wood Waste

Margareta Novian Cahyanti, Sabarathinam Shanmugam * and Timo Kikas * 

Chair of Biosystems Engineering, Institute of Forestry and Engineering, Estonian University of Life Sciences, Kreutzwaldi 56, 51014 Tartu, Estonia; margareta.cahyanti@emu.ee

* Correspondence: sabarathinam.shanmugam@emu.ee (S.S.); timo.kikas@emu.ee (T.K.)

Abstract: Abundant availability of lignocellulosic biomass (LCB) coupled with diverse pretreatment methods have made it a promising option for energy production. However, it faces several challenges, some of which can be overcome by integrating pretreatment processes. The present study aims to optimize the integration of two different pretreatment methods—torrefaction (to reduce moisture content and fractionate biomass) and alkaline pretreatment of wood waste (to delignify biomass)—and utilize it for bioethanol production. Pretreatment performance was evaluated based on delignification, biomass hydrolysis, and bioethanol production. Initially, torrefaction was performed in a continuous reactor at a temperature range of 225–300 °C, followed by optimization of the critical parameters of alkaline pretreatment of torrefied wood waste (TWW), that is, the temperature, reaction time, solid–liquid ratio, and alkali concentration. Subsequently, the chemical and carbohydrate compositions of raw wood waste (RWW) and TWW were studied, followed by enzymatic hydrolysis and bioethanol fermentation. Integrated pretreatment positively impacted the cellulose and glucose contents of raw and torrefied biomass at lower temperatures. The enzymatic hydrolysis of TWW treated with alkali produced higher levels of glucose and bioethanol than (stand-alone) TWW. These results can be used as a basis for choosing the most suitable pretreatment for enhanced biomass conversion.

Keywords: integrated pretreatment; torrefaction; alkali delignification; enzymatic hydrolysis; cellulose



Citation: Cahyanti, M.N.; Shanmugam, S.; Kikas, T. Synergistic Effects of Torrefaction and Alkaline Pretreatment on Sugar and Bioethanol Production from Wood Waste. *Energies* **2023**, *16*, 7606. <https://doi.org/10.3390/en16227606>

Academic Editor: Fabio Montagnaro

Received: 24 October 2023

Revised: 10 November 2023

Accepted: 13 November 2023

Published: 16 November 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Biomass has attracted significant attention as a renewable energy source, owing to its abundant availability. It can be sourced from forestry crops, agricultural residues, and industrial wastes. Forestry residues, in particular, exhibit a high cellulose content, making them excellent candidates as substrates for energy production. Hardwood, for instance, contains 40–50% cellulose, whereas softwood has a cellulose content of 45–50% [1]. In contrast, agricultural waste, such as wheat straw, has a lower cellulose content, typically ranging from 33 to 40% [2]. However, utilizing wood waste for energy production is challenging, primarily owing to its elevated moisture content. The substantial moisture content renders biomass susceptible to microbial degradation and increases transportation costs [3]. To address these issues, biomass necessitates pretreatment, and torrefaction has emerged as a viable option to reduce water content, a task conventional drying struggles to accomplish [4].

Torrefaction, a thermochemical treatment conducted within the temperature range of 200–300 °C in an inert atmosphere, induces the thermal degradation of hemicellulose, which is rich in hydrophilic hydroxyl groups [5]. Consequently, torrefaction increases the content of cellulose and lignin products with enhanced hydrophobic properties. According to Xin et al. [6], torrefied biomass (TB) exhibits reduced moisture absorption compared to its raw counterpart. After torrefaction at 290 °C, the moisture absorption capacity of herbal medicine waste decreased to 15%, whereas raw herbal medicine waste absorbed as much as 24%. TB is more hydrophobic than raw biomass. It also has a higher bulk density, which reduces transportation costs [7].

Despite its positive effects on biomass fuel characteristics, torrefaction has traditionally been employed solely as a precursor to the combustion and gasification processes. However, there is a compelling need for in-depth investigation into the application of torrefaction as a pretreatment in biochemical conversion processes, especially those related to fermentable sugar production. There are contrasting reports on glucose yield after enzymatic hydrolysis of TB. Previous studies by Chiaramonti et al. [8] found that torrefaction may reduce glucose yield during enzymatic hydrolysis. Conversely, Chaluvadi et al. [9] and Sheikh et al. [10] suggested that glucose levels increased with torrefaction temperature until they reached a peak, after which they tended to decline. The disparity in enzymatic hydrolysis yield is due to the formation of acids and inhibitors of hemicellulose and lignin at higher temperatures [11]. In addition, carbohydrate degradation above 270 °C results in pseudo-lignin formation with altered surface properties that display a higher affinity toward saccharifying enzymes due to non-specific adsorption [12]. Normark et al. [13] reported a similar observation of decreased carbohydrate content in spruce under mild torrefaction conditions with an increased Klason lignin content that contributed to reduced glucose yield.

Furthermore, an increase in torrefaction temperature severely affects the molecular architecture of lignin via a condensation reaction that results in both an increase in phenolic groups and hydrophobicity, which also inhibits the enzymatic hydrolysis of cellulose [14,15]. Hence, removing lignin before enzymatic hydrolysis can significantly enhance enzymatic saccharification and increase the glucose yield. Although there has been extensive research on lignin extraction from raw biomass, few studies have focused on extracting lignin from TB, specifically from wood waste. Thus, the present study aimed to (i) optimize lignin extraction from torrefied wood waste (TWW) at different temperatures, (ii) characterize the resulting delignified biomass, (iii) quantify glucose and inhibitor production during enzymatic hydrolysis, and (iv) utilize the generated glucose in the bioethanol production process. This study seeks to address critical gaps in our understanding of the potential application of torrefaction as a pretreatment in biochemical conversion processes, ultimately advancing biomass utilization for sustainable energy production.

2. Materials and Methods

2.1. Materials

The feedstock used in this study was wood waste supplied by Sarles OÜ in Tartu, Estonia, consisting of spruce, birch, and pine chips. The wood waste was milled using a Retsch SM100 mill (Retsch GmbH, Haan, Germany). Before the experiment, it was air-dried for approximately two weeks. The hydrolytic enzyme cocktail ACCELLERASE 1500 (isolated from *Trichoderma reesei*) was purchased from Genencor, Inc. (Palo Alto, CA, USA).

2.2. Torrefaction

The torrefaction procedures were conducted following the methodology established in a previous study by [16]. Torrefaction occurred within a continuous torrefaction reactor system, and an inert environment was maintained using nitrogen as the carrier gas. Torrefaction was performed at three distinct temperatures, 225 °C, 275 °C, and 300 °C, each for 60 min. Subsequently, the resulting TB was cooled, collected, ground, and stored in air-tight plastic containers to facilitate further analysis.

2.3. Alkali-Mediated Pretreatment

Delignification of the raw wood waste (RWW) and TWW was performed using alkali (NaOH) pretreatment. The process parameters of the alkali pretreatment, that is, the alkali concentration [1M–4M], reaction temperature (60–100 °C), residence time (60–300 min), and biomass-to-liquid ratio (1:10–1:50 (*w/v*)), were optimized to enhance lignin removal. After pretreatment, the mixture was separated with centrifugation at 4500 rpm for 5 min, and the supernatant was collected. The biomass was subsequently washed with 50 mL of distilled water (thrice), and the resulting liquid fraction was collected. The supernatant

and liquid fraction were combined, and the lignin was precipitated by adjusting the pH to 2. Subsequently, the lignin and biomass were separated with centrifugation at 4500 rpm for 5 min. The lignin and biomass fractions were separately washed until they reached a neutral pH and dried at 60 °C. The yields of polysaccharides and lignin recovered after pretreatment were calculated using the following equations:

$$\text{Polysaccharides yield(\%)} = \frac{\text{Mass of biomass after alkali pretreatment}}{\text{Mass of biomass before alkali pretreatment}} \times 100\% \quad (1)$$

$$\text{Lignin recovery(\%)} = \frac{\text{Mass of dried lignin}}{\text{Mass of biomass}} \times 100\% \quad (2)$$

2.4. Enzymatic Hydrolysis

Enzymatic hydrolysis was conducted based on earlier research by Rooni et al. [17] with some modifications. Enzymatic hydrolysis was performed in a 100 mL screw-cap bottle at 50 °C for 48 h in an orbital shaker (220 rpm). Briefly, 0.75 g of delignified biomass was added to each bottle, followed by 15 mL of citrate buffer (0.005 M) to maintain a pH of 4.8. The enzyme cocktail (ACCELLERASE 1500) was utilized at a ratio of 0.3 mL per gram of dry biomass. Following enzymatic hydrolysis, the resulting hydrolysate was separated with centrifugation at 4500 rpm for 5 min. The solid biomass was washed with 5 mL of buffer, and the liquid fraction was collected for fermentation.

2.5. Bioethanol Fermentation

Yeast obtained (Superjæst T3) from a local market was used for the ethanol fermentation. For activation, one gram of yeast was added to an activation medium composed of 10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose, and the mixture was incubated at 32 °C for 24 h at 150 rpm in an orbital shaker. For the fermentation, the hydrolysate obtained from the enzymatic hydrolysis was enriched with 1.0 g/L yeast extract, 1.6 g/L NH₄Cl, and 1.0 g/L of MgSO₄. For 10 mL hydrolysate, 1 mL activated yeast was added. The flask was incubated in the orbital shaker at 32 °C for 24 h at 150 rpm. After fermentation, the liquid fraction was separated with centrifugation at 4500 rpm for 5 min and analyzed for ethanol production using an HPLC LC—2030C 3D Plus (Shimadzu Corporation, Kyoto, Japan) [17].

2.6. Characterization of Pretreated Biomass

2.6.1. Fiber Analysis

The fiber content of the raw, torrefied, and delignified wood waste was determined using an Ankom 200 fiber analyzer (ANKOM Technology, Fairport, NY, USA), and the fiber composition was determined following the methodology outlined by [16].

2.6.2. Ash Content

To quantify the ash content, a test sample of 1–2 g of biomass was placed in the Milestone PYRO microwave muffle furnace at a temperature of 550 °C for a period of 4 h in accordance with the protocol outlined in CEN/TS 14775:2004 [18].

2.6.3. FTIR Analysis

The surface functionalities of non-delignified and delignified biomass were analyzed using FTIR spectroscopy (Spectrum BXII, Perkin Elmer Inc., Waltham, MA, USA) employing the universal attenuated total reflection (ATR) method. The spectra, recorded at a resolution of 4 cm^{−1}, covered the 4000–600 cm^{−1} interval range with an average accumulation of 16 scans.

2.6.4. Sugar Composition

The NREL procedure was used for the sugar composition analysis [19]. The experimental procedure consists of two separate phases. During the first stage of the experiment, a concentrated acid solution (72% H₂SO₄) was used at a relatively low temperature (30 °C) for 1 h. Subsequently, in the second stage, the acid solution was diluted to 3%, and the reaction vessels were heated to 121 °C in an autoclave for 1 h.

2.6.5. HPLC Analysis

Sugars, inhibitors, and ethanol were determined according to the method outlined by [20]. Before the analysis, the samples were centrifuged at 10,000 rpm for 5 min (twice), followed by filtration using centrifugal filters (0.2 µm) at 10,000 rpm for 5 min. For sugar content, an Aminex HPX-87P column with water as the mobile phase was used at 0.6 mL/min with column and detector (Shimadzu RID-20A) temperatures at 85 °C and 65 °C, respectively. For inhibitors and ethanol content, a RezexTM ROA-Organic Acid H⁺ column with a 5 mM sulfuric acid mobile phase was used at 0.5 mL/min with both column and detector at 50 °C.

2.7. Statistical Analysis

The experiments were conducted in triplicate, and the data are expressed as the mean ± standard deviation.

3. Results and Discussion

3.1. Optimization of Process Parameters in Alkaline Pretreatment

The increased lignin content in TB poses a significant barrier to enzymatic cellulose hydrolysis, as hydrolytic enzymes exhibit a stronger affinity for lignin than for cellulose glycosidic bonds [21]. Therefore, reducing the lignin content in TB is crucial for its use as a feedstock for fermentation. Alkaline pretreatment is primarily employed to extract lignin and enhance the polysaccharide yield [22]. Thus, the influence of operational parameters (residence time, temperature, solid–liquid ratio, and alkaline concentration) in alkaline pretreatment on lignin extraction, polysaccharide recovery, and mass loss was investigated using a one-factor-at-a-time approach.

3.1.1. Effect of Alkali Concentration and Solid–Liquid Ratio on Lignin Extraction

Alkali (NaOH) concentration plays a critical role in lignin extraction from LCB [23]. Initially, a range of NaOH concentrations from 1M to 4M was investigated to determine the optimal NaOH concentration for maximizing lignin extraction (Figure 1a). Under these optimal alkali concentrations, the lignin extraction rates were 57.82% for RWW and 17.3%, 33.5%, and 18.3% for TWW (T₂₂₅, T₂₇₅, and T₃₀₀, respectively). Furthermore, optimal NaOH extraction improved polysaccharide yields of 67.91%, 72.49%, 74.71%, and 76.08% for untreated and TWW (T₂₂₅, T₂₇₅, and T₃₀₀), respectively. Compared to RWW, TWW exhibited enhanced polysaccharide retention, emphasizing the significance of employing an optimal alkali concentration to boost lignin extraction and polysaccharide recovery [24]. Additionally, the impact of the solid-to-liquid ratio on lignin extraction was explored from 1:10 to 1:40 (Figure 1b). Notably, untreated RWW and TWW at different temperatures exhibited varying solid-to-liquid ratio requirements. Specifically, RWW required a 1:10 ratio. In contrast, biomass torrefied at T₂₂₅ required a 1:30 ratio to extract 15.78% of lignin, whereas biomass torrefied at T₂₇₅ and T₃₀₀ required a 1:20 ratio to remove 26.8% and 20.4% of lignin, respectively. The higher biomass-to-alkali ratio observed in TB at elevated torrefaction temperatures may be attributed to the increased complexity of biomass composition [16]. This complexity, in turn, necessitates more alkali for efficient lignin extraction from the biomass.

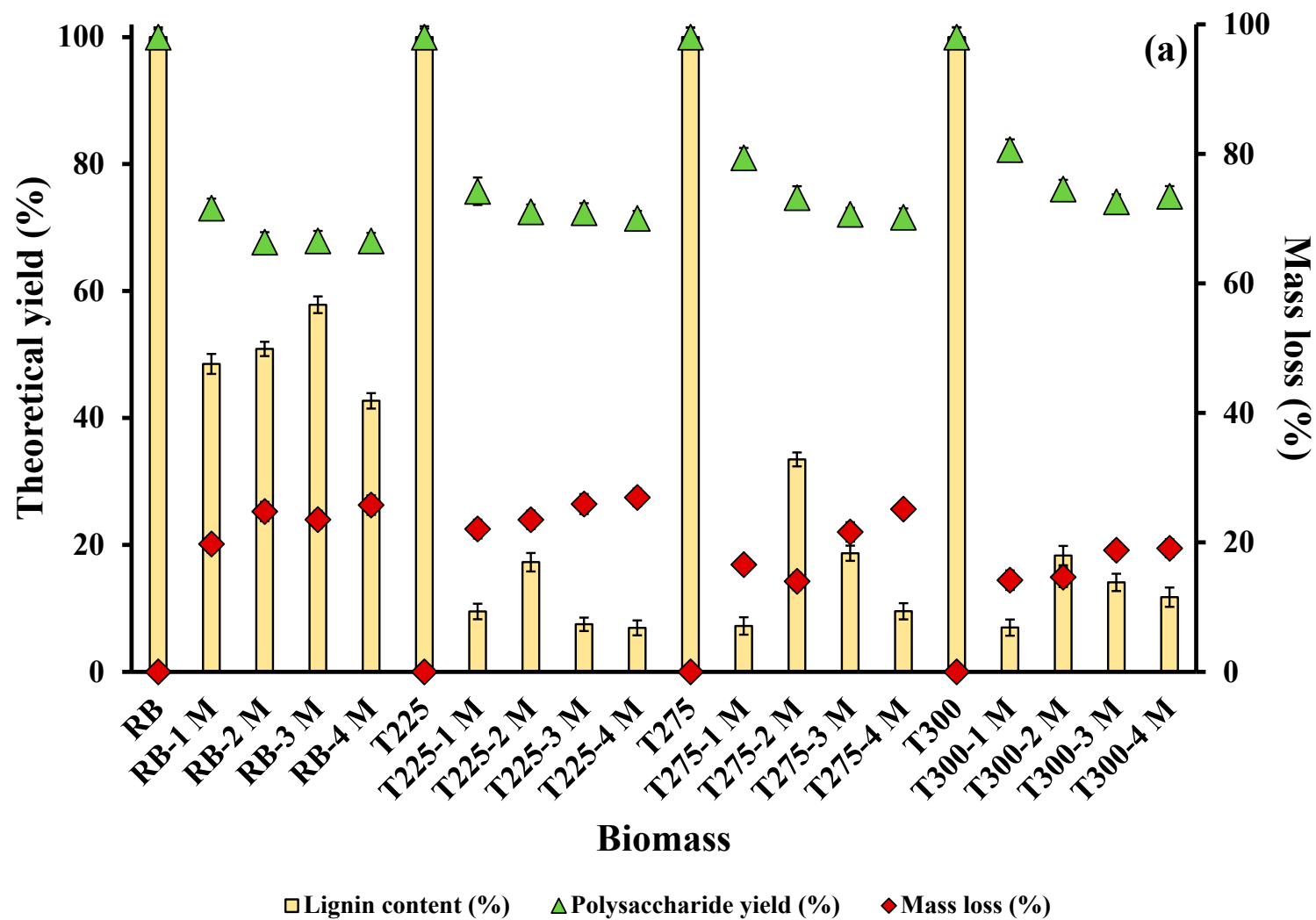


Figure 1. Cont.

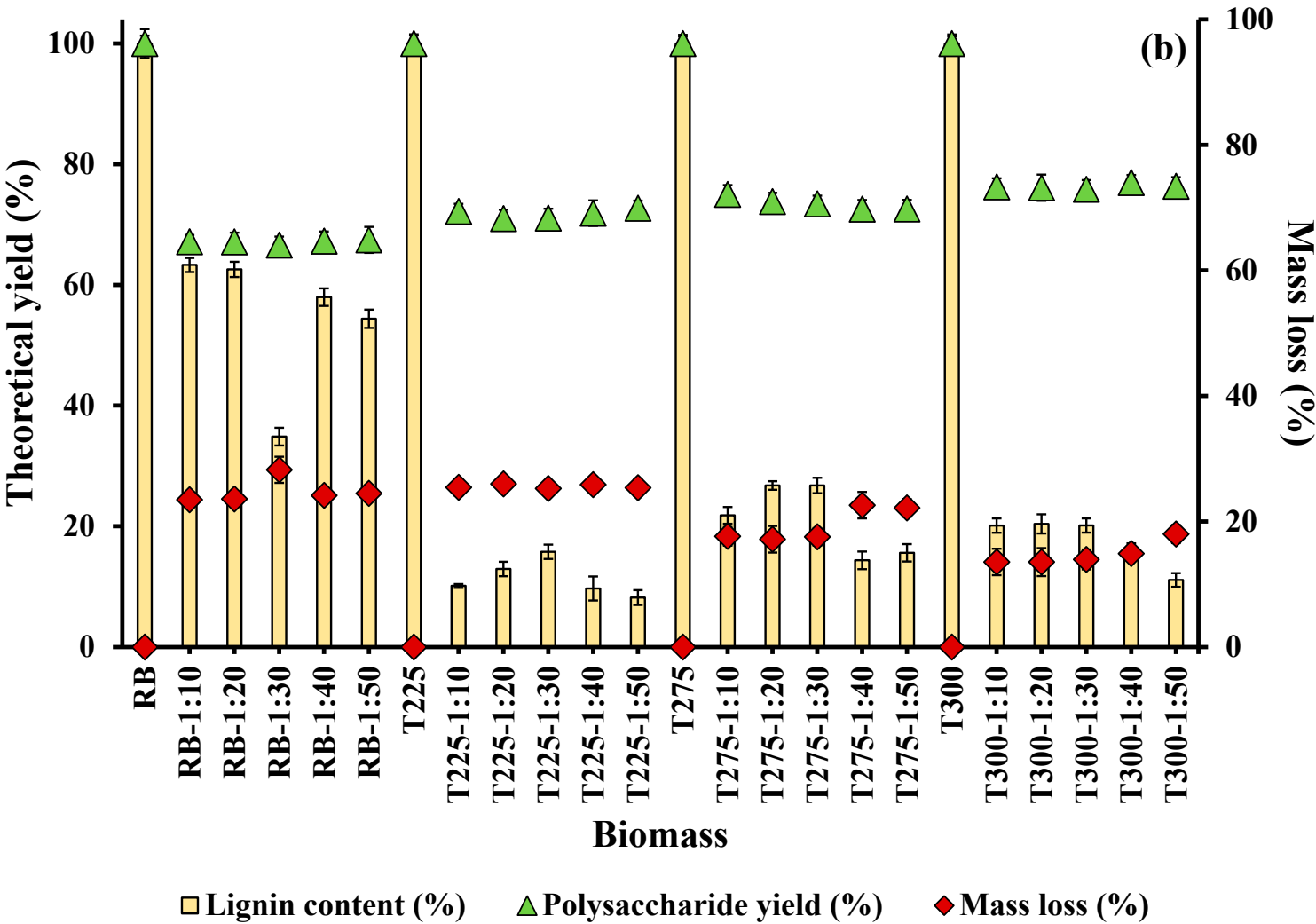


Figure 1. Cont.

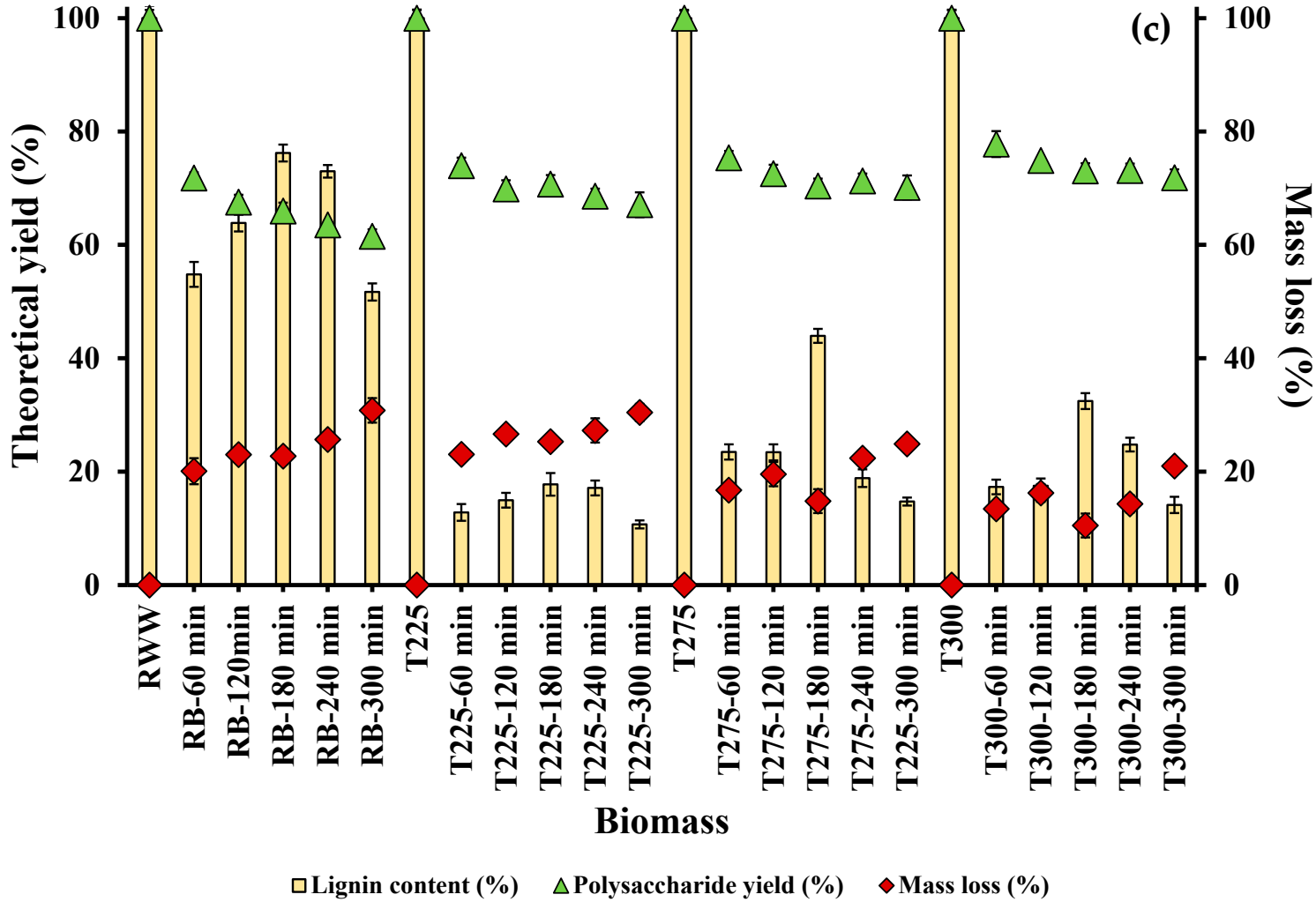


Figure 1. Cont.

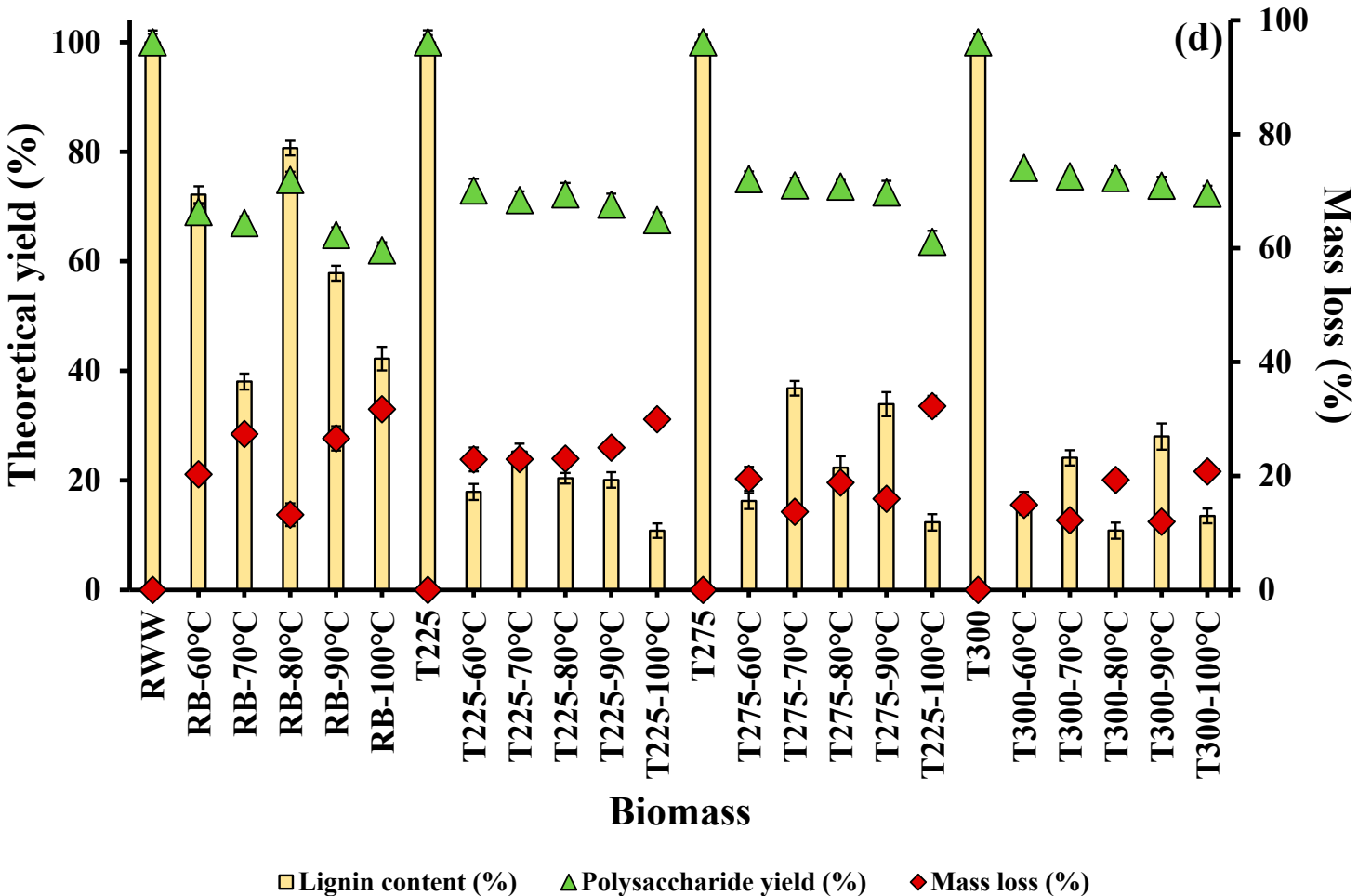


Figure 1. Optimization of process parameters for delignification of raw and torrefied wood waste using alkaline pretreatment. (a) alkali concentration; (b) solid-to-liquid ratio; (c) residence time; (d) temperature.

3.1.2. Effect of Residence Time on Lignin Extraction

The effects of various residence times (60–300 min) were studied at a constant temperature of 80 °C with an alkali concentration of 2M and a solid-to-liquid ratio of 1:30 (Figure 1c). From the results, it was observed that lignin extraction increased with increasing incubation time, and maximal lignin extraction was attained at 180 min for both RWW (76.2%) and TWW biomass (T_{225} (17.1%), T_{275} (43.9%), T_{300} (32.4%)). Increasing the incubation time beyond this period did not significantly improve lignin extraction. However, an increase in the residence time reduced polysaccharide yield and increased the mass loss of the pretreated biomass. At 180 min, the raw, T_{225} , T_{275} , and T_{300} samples obtained polysaccharide yields of 65.9%, 70.7%, 70.4%, and 73.1%, respectively. Moreover, the mass loss ranges of the RWW, T_{225} , T_{275} , and T_{300} biomass at 180 min were 22.74%, 25.29%, 14.8%, and 10.5%, respectively. The increase in lignin yield, mass loss, and polysaccharide yield indicated that an increased residence time had a profound effect on solubilizing lignin, which also had a limited impact on solubilizing cellulose and hemicellulose (RWW) during alkali pretreatment [25].

3.1.3. Effect of Temperature on Lignin Extraction

The optimum incubation temperature was determined based on its impact on lignin extraction. Various temperatures, ranging from 60 °C to 100 °C, were investigated (Figure 1d), which resulted in lignin extractions of 80.69% (80 °C), 25.24% (70 °C), 36.83% (70 °C), and 28.01% (90 °C) for RWW, T_{225} , T_{275} , and T_{300} TWW, respectively. The optimal temperature retained a higher percentage of polysaccharides (74.9%, 71.2%, 73.9%, and 73.8%) and reduced mass loss (13.20%, 22.96%, 13.71%, and 11.97%) in T_{225} , T_{275} , and T_{300} TWW, respectively. This improved polysaccharide recovery was attributed to enhanced lignin extraction from the raw and pretreated biomass at elevated temperatures. These findings align with those of Corderi et al. [26], who reported that lignin extraction at higher temperatures increased polysaccharide yield from mixed hardwood biomass.

Under these optimized conditions, alkaline pretreatment achieved maximum lignin extractions of 77.2%, 30.2%, 34.4%, and 20.7% for RWW, T_{225} , T_{275} , and T_{300} TWW, respectively. TB exhibited an increasing trend in polysaccharide retention and reduced mass loss compared to RWW, demonstrating the synergistic effect of alkali pretreatment on polysaccharide extraction. A similar observation was made by Tripathi et al. [27], where the impact of alkaline pretreatment on the torrefaction of wheat straw significantly enhanced the polysaccharide yield. However, their research typically involved the alkaline pretreatment of grassy biomass followed by torrefaction. To the best of our knowledge, this study represents the first report of the synergistic application of integrated pretreatment for TB, enabling lignin extraction followed by polysaccharide extraction for fermentation.

3.2. Effect of Integrated Pretreatment on Biomass Composition and Properties

3.2.1. Chemical Composition of Biomass

The effects of torrefaction and alkali pretreatment on the chemical composition of the biomass are shown in Figure 2. Compared to RWW, the hemicellulose content of TWW at 225 °C decreased by 27.2% and was undetectable after torrefaction at 275 °C and 300 °C. As the most reactive fiber in LCB, hemicellulose easily decomposes at a temperature range of 220–315 °C [28]. The reduction in hemicellulose content in the biomass increased as the torrefaction severity index increased, which aligns well with the early report of Cahyanti et al. [16]. After alkali treatment, the hemicellulose content of RWW decreased to 16.2%. Compared to TB (T_{225}), delignified TB (DT_{225}) showed a moderate reduction (7%) in the hemicellulose content. The reduction in the hemicellulose content after alkali pretreatment of LCB and TB is due to the cleavage of ester bonds between the saponified hemicellulose and lignin moieties [29].

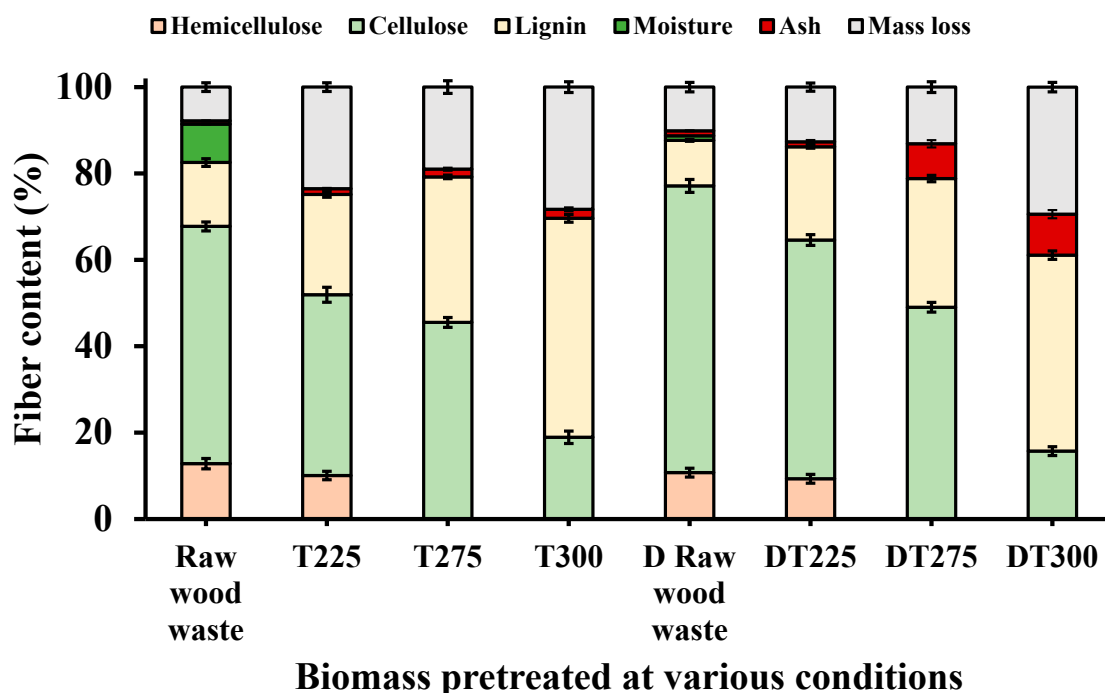


Figure 2. Biomass composition of raw and torrefied wood waste before and after alkali treatment.

The cellulose content in the RWW was initially 55%. Following torrefaction at 225 °C, the cellulose content of the RWW decreased to 41.9% (76.2%) and sharply dropped to 18.9% (34.4%) after torrefaction at 300 °C. The reduction in the cellulose content of RWW can be attributed to the initiation of cellulose degradation at 200 °C with the degradation rate increasing at elevated reaction temperatures [30]. These findings are consistent with those of Cahyanti et al. [16] in which the cellulose content decreased from 54% to 43% after torrefaction at 225 °C and, eventually, reached 31% after torrefaction at 300 °C for 60 min. After alkali pretreatment, the cellulose content increased for all biomass types except for the TB at 300 °C. For example, in the case of biomass torrefied at 225 °C, the cellulose content increased from 41.9% to 55.3%. The increase in cellulose content is a result of lignin and hemicellulose degradation during the pretreatment process, leading to a higher proportion of cellulose in the biomass [31]. Kathirselvam et al. [32] observed an increase in cellulose content following alkali treatment with *Thespesia populnea* showing an increase from 70% to 76% cellulose content after treatment with 5% sodium hydroxide.

After torrefaction, the lignin content in the biomass tended to increase based on fiber analysis. In the RWW, the lignin content was 14.8%, which increased to 23.2%, 33.7%, and 51% at 225, 275, and 300 °C, respectively, after torrefaction. The elevated level of lignin is probably due to the formation of pseudo-lignin, a byproduct of degraded polysaccharides (cellulose and hemicellulose) that are generated under severe pretreatment conditions [20]. Furthermore, a similar increase in lignin content with increasing torrefaction temperature has been reported for woody biomass, agricultural reuse, and wood waste [13,16]. After alkali treatment, the lignin content in the TB at 300 °C decreased from 51% to 45%, owing to swelling of the fibers in the RWW, leading to the formation of a larger surface area for the action of OH^- ions on the ferulic acid linkages of lignin, which aids in improving the delignification process [33]. Furthermore, increasing the temperature of the torrefaction process led to increased ash content in both TB and alkali-treated TB. The release of volatile matter at higher temperatures results in the condensation of solid materials, which eventually increases the ash content, similar to the earlier report of Cahyanti et al. [16]. In addition, the increase in mass loss with increased torrefaction temperature is also in accordance with Tripathi et al. [27] for torrefaction and alkaline pretreatment of wheat straw.

3.2.2. Sugar Composition of Biomass

Figure 3 shows the changes in glucose, mannose, and xylose levels before and after alkali pretreatment under the chosen conditions for all biomasses. The glucose content was reduced (22%) after torrefaction at 225 °C. The reduction after torrefaction is related to the degradation of hemicellulose and cellulose as sources of glucose. However, the glucose content of T₂₇₅ increased to 27% compared with that of T₂₂₅ because of the fragmentation of hemicellulose via aromatization and crosslinking of degraded sugars [34]. After alkali treatment, the glucose content increased, except in T₃₀₀. In a previous study by Noori and Karimi [35], an increase in glucan content was observed after alkali treatment with the glucan content of pine increasing from 35% to 44% following treatment with alkali. An increase in the glucan content from 44% to 54% has also been observed in poplars after alkali treatment at 93 °C [36]. A reduction in mannose was observed after torrefaction at 225 °C, and complete removal was observed at higher torrefaction temperatures, similar to a previous report on the reduction in mannose at higher torrefaction temperatures [37]. The reduction after torrefaction is related to hemicellulose degradation because mannose sugars are derived from hemicellulose [38]. The reduction in mannose concentration prevailed after alkali pretreatment due to the reduction in the hemicellulose content of TWW. Hu et al. [39], observed a similar pattern with the removal of most of the mannose from wheat straw when treated with 1% NaOH. A reduction in xylose was observed after torrefaction at 225 °C but was undetectable in T₂₇₅ and T₃₀₀. The reduction after torrefaction was related to hemicellulose degradation. After alkaline treatment, the mannose and xylose contents were reduced. A decrease in xylan content after alkaline treatment was also observed in a previous study by Noori and Karimi [35], who stated that xylan content from pine decreased from 7.5% to 5.6% after treatment with alkali at 80 °C. A decrease in xylan content from 25% to 11% has also been observed in poplars after alkali treatment at 93 °C [36].

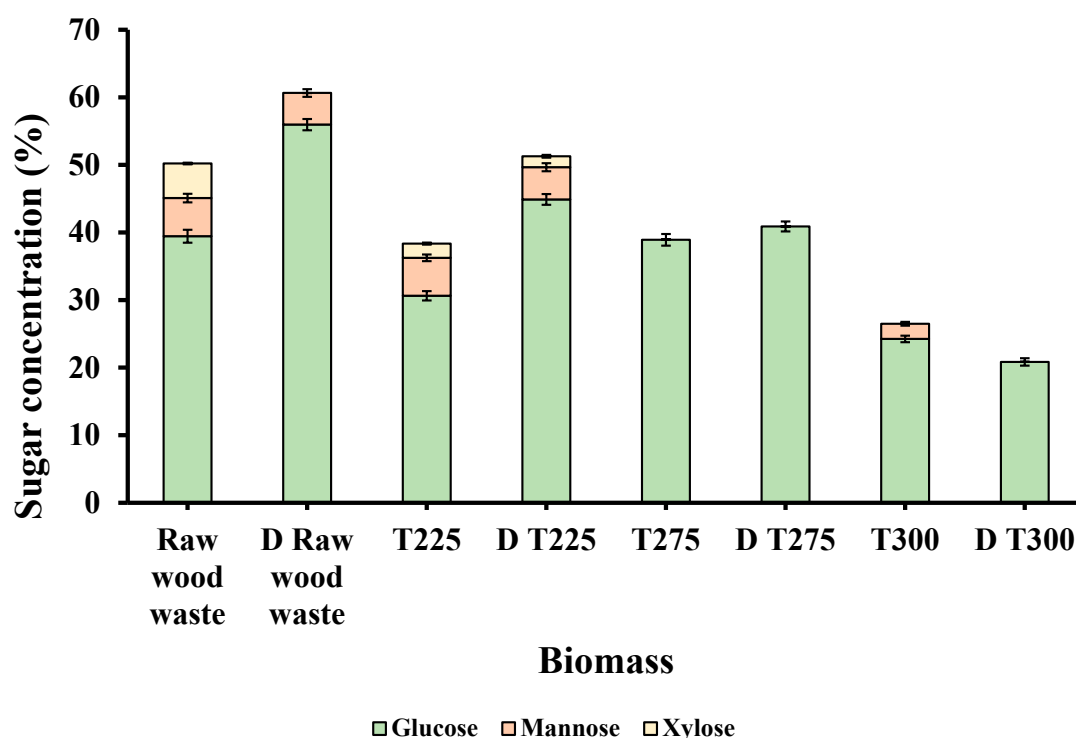


Figure 3. Sugar composition of raw and torrefied wood waste before and after alkali treatment.

3.2.3. Impact of Pretreatment on Cellulose and Lignin-Related Characteristics

The chemical modifications induced by the torrefaction and alkaline pretreatment of RWW and TWW were determined using FTIR analysis (Figure 4). FTIR analysis facilitates the assessment of cellulose crystallinity, bound water content, and lignin deformation by

employing the lateral order index (LOI), hydrogen bond intensity (HBI), and cross-linked lignin (CLL) [40]. The empirical crystallinity of cellulose, described by LOI, is defined as the ratio between the β -(1,4) glycosidic bond in cellulose (897 cm^{-1}) and the crystalline structure of cellulose (1423 cm^{-1}). The LOI of untreated raw wood waste was similar to that of torrefied and delignified TB at $225\text{ }^{\circ}\text{C}$ (Table 1). However, biomass subjected to higher torrefaction temperatures ($275\text{ }^{\circ}\text{C}$ and $300\text{ }^{\circ}\text{C}$) and their corresponding delignified counterparts exhibited increased LOI values. This suggests the presence of more ordered and crystalline cellulose [41], which aligns with prior research that indicated that elevated torrefaction temperatures lead to higher cellulose crystallinity, as observed by Lin et al. [42]. Moreover, the heightened cellulose crystallinity and improved structural organization resulting from torrefaction are substantiated by an increased HBI index. This index was calculated based on the ratio between the C–H rocking of the glucose ring (at 3400 cm^{-1}) and the O–H stretching originating from hydrogen bonds between the molecules (at 1320 cm^{-1}). With elevated torrefaction temperatures, there was an increasing trend in HBI for TWW and delignified TWW (DTWW). This phenomenon can be attributed to the increased inter- and intra-chain hydrogen bonds between adjacent cellulose chains, resulting in more condensed crystalline cellulose structures [43]. The impact of pretreatment on lignin condensation and the formation of cross-linked structures, particularly the guaiacyl concentration of polysaccharides during torrefaction and alkaline pretreatment, was assessed using CLL. This assessment was performed by calculating the ratio of the deformation of lignin [CH_2 and CH_3] at 1508 cm^{-1} and the stretching of aromatic lignin [$\text{C}=\text{C}$ and $\text{C}=\text{O}$] at 1600 cm^{-1} [40]. Similar to LOI and HBI, an increased CLL was observed at higher torrefaction temperatures. This suggests that, in TWW-delignified torrefied soil, lignin recondensation increases with increasing torrefaction temperature [14]. Conversely, in alkaline pretreatment, there is evidence of lignin repolymerization or recondensation on the cellulose surface, leading to cross-linkages between polysaccharides and residual lignin [44]. FTIR analysis revealed that elevated torrefaction temperatures significantly impacted biomass composition and structure, potentially influencing subsequent hydrolysis and microbial fermentation processes.

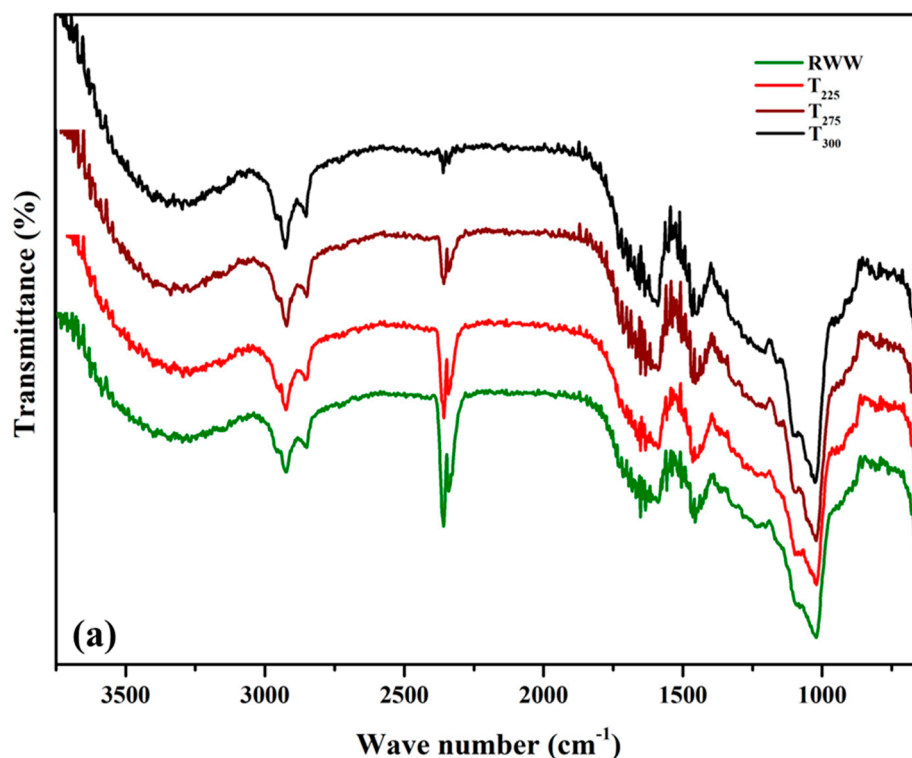


Figure 4. Cont.

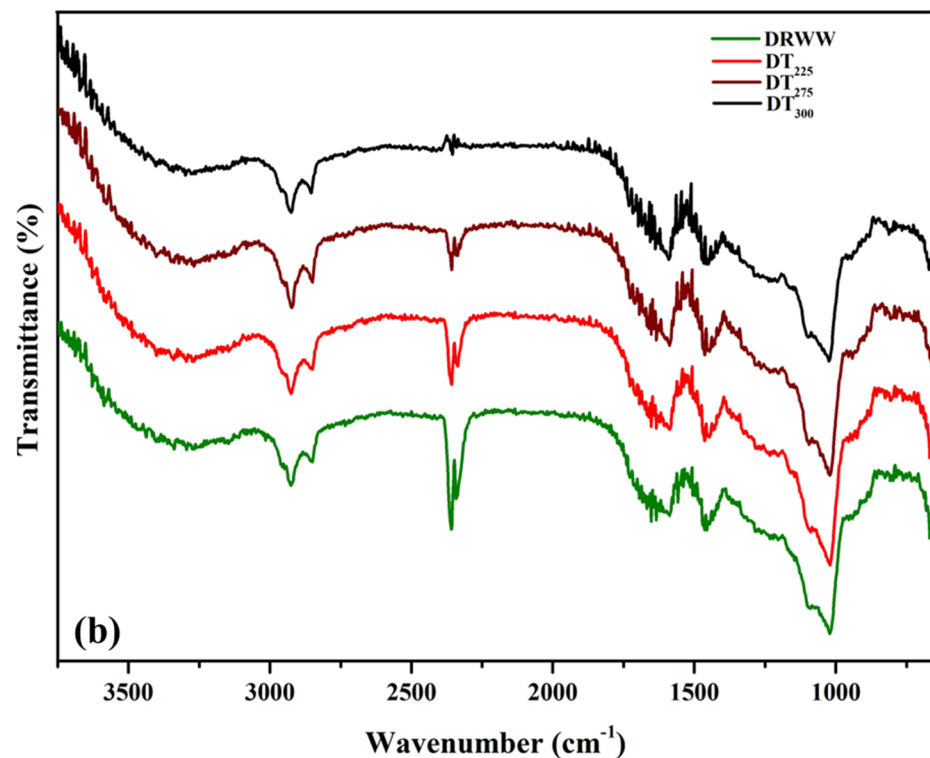


Figure 4. AT-FTIR spectra of torrefied biomass before (a) and after (b) alkali pretreatment.

Table 1. Impact of pretreatment on cellulose and lignin-related characteristics.

Biomass	LOI (A_{1423}/A_{897})	HBI (A_{3400}/A_{1323})	CLL (A_{1508}/A_{1600})
Raw wood waste	0.98 ± 0.003	2.20 ± 0.001	1.13 ± 0.004
Torrefied wood waste			
T ₂₂₅	0.97 ± 0.007	2.21 ± 0.005	1.15 ± 0.007
T ₂₇₅	1.20 ± 0.005	2.85 ± 0.004	1.23 ± 0.003
T ₃₀₀	1.00 ± 0.008	2.97 ± 0.007	1.20 ± 0.007
Delignified torrefied wood waste			
DT ₂₂₅	0.99 ± 0.008	2.21 ± 0.007	1.16 ± 0.006
DT ₂₇₅	1.09 ± 0.006	2.55 ± 0.004	1.18 ± 0.005
DT ₃₀₀	1.14 ± 0.007	2.64 ± 0.008	1.24 ± 0.008

LOI, lateral order index; HBI, hydrogen bond intensity; CLL, cross-linked lignin. The data presented are the means $\pm$ standard deviation ($n \geq 3$).

3.3. Enzymatic Hydrolysis

The sugar content and composition of liquid hydrolysates are critical factors for fermentation applications. The glucose, mannose, and xylose concentrations obtained after the enzymatic hydrolysis are shown in Figure 5. In this study, enzymatic hydrolysis was performed separately before fermentation, and the concentration of glucose produced from the TWW was lower than that produced from the RWW. The glucose concentration in the RWW was 2.74%, whereas that in the TWW was 1.39% (T₂₂₅) and 0.85% (T₂₇₅) and decreased to 0.64% (T₃₀₀). These results are in comparison with those of Tripathi et al. [27], where the glucose concentration in the hydrolysate was reduced from 27.7% (raw biomass) to 2.4% and 1.5% for wheat straw torrefied at 220 °C and 240 °C, respectively. In contrast, the mannose and xylose concentrations in the hydrolysate from the T₂₂₅ biomass (0.38% and 0.8%, respectively) were slightly higher than those in the raw biomass (0.62% and 0.41%, respectively), which can be attributed to the degradation of polysaccharides. Furthermore, mannose and xylose were not detected in the hydrolysate of TB at higher temperatures (T₂₇₅ and T₃₀₀). The reduction in monosaccharides occurred because of the

degradation of cellulose and hemicellulose as sources of glucose, mannose, and xylose. Furthermore, the higher cellulose crystallinity and hydrophobicity attained at higher torrefaction temperatures severely inhibit hydrolytic enzyme access to cellulose, resulting in reduced saccharification [7,27].

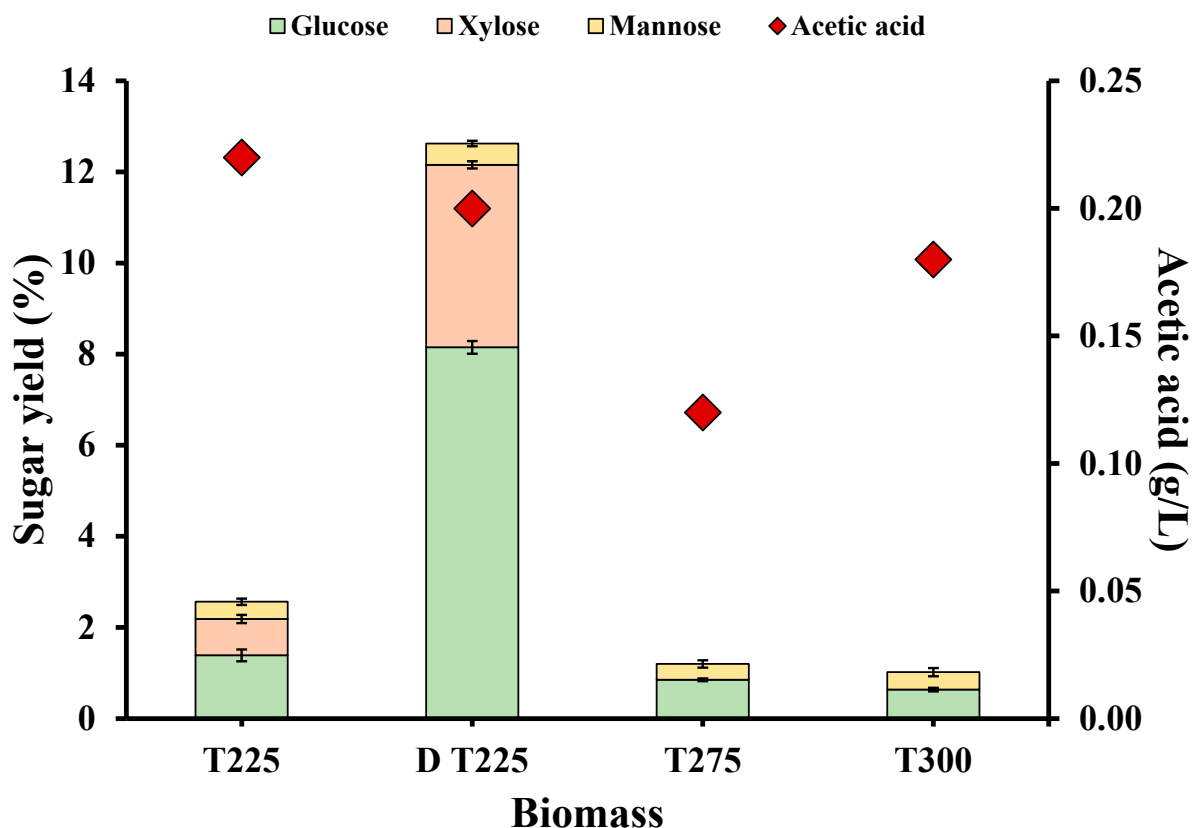


Figure 5. Sugar and acetic acid concentrations of raw and torrefied biomass after enzymatic hydrolysis.

However, alkaline pretreatment significantly improved the glucose concentration in both the RWW and TWW (T_{225}), whereas the glucose concentration was not detected at higher temperatures (T_{275} and T_{300}). The glucose concentration in the RWW increased from 2.74% to 43.30% after alkali treatment, and in the DT_{225} , the glucose concentration increased almost six-fold (8.15%) compared to that of T_{225} . Alkali treatment had a similar effect on the xylose concentration in the hydrolysate, where the DT_{225} xylose concentration of 4.01% is significantly higher than that of T_{225} (0.80%). Alkali treatment releases the lignin from the biomass, reduces the intense complexity of the biomass fiber, and, finally, increases the access for an enzyme to cellulose and hemicellulose. These observations are similar to those reported by Noori and Karimi [35] and Kim [45], who found a significant increase in the concentrations of glucose and xylose after alkali pretreatment.

Acetic acid, a major inhibitor of hemicellulose degradation during torrefaction, is known for its profound inhibitory effect on yeast growth and subsequent glucose conversion rate to ethanol. As shown in Figure 5, the acetic acid content in the hydrolysate from the biomass before alkali treatment ranged from 0.12 to 0.22 g/L. The hydrolysate from the biomass torrefied at high temperatures had a higher acetic acid content, presumably due to the amount of acetic acid recondensed during the torrefaction process. However, the concentrations obtained for the RWW torrefied at different temperatures (225 °C, 275 °C, and 300 °C) are lower than the acetic acid content in the hydrolyzate of wheat straw, which ranged from 0.64 to 0.69 g/L [46]. After alkali treatment, the acetic acid content of the hydrolysate was reduced. This phenomenon occurred because of the degradation of hemicellulose, and the washing process after the alkali treatment eliminated the acetic acid content in the TB [47].

3.4. Bioethanol Production

Bioethanol production from the RWW and TWW after 44 h of fermentation is shown in Figure 6. The RWW without alkali treatment produced 24 g/kg bioethanol. This value is close to the value obtained by Salehian and Karimi [48], who used pine branches as feedstock for bioethanol production (24 g/kg). Bay et al. [36] used pine as feedstock and obtained 26 g/kg of bioethanol. The bioethanol yield of the TWW was lower than that of the RWW (21.7 g/kg). This was due to the lower glucose content in the hydrolysate from TB. The increase in ethanol production in raw wood was higher than that in TB, which could be due to the higher acetic acid content of the hydrolysate that triggered stress in the inoculum and suppressed glucose conversion to ethanol.

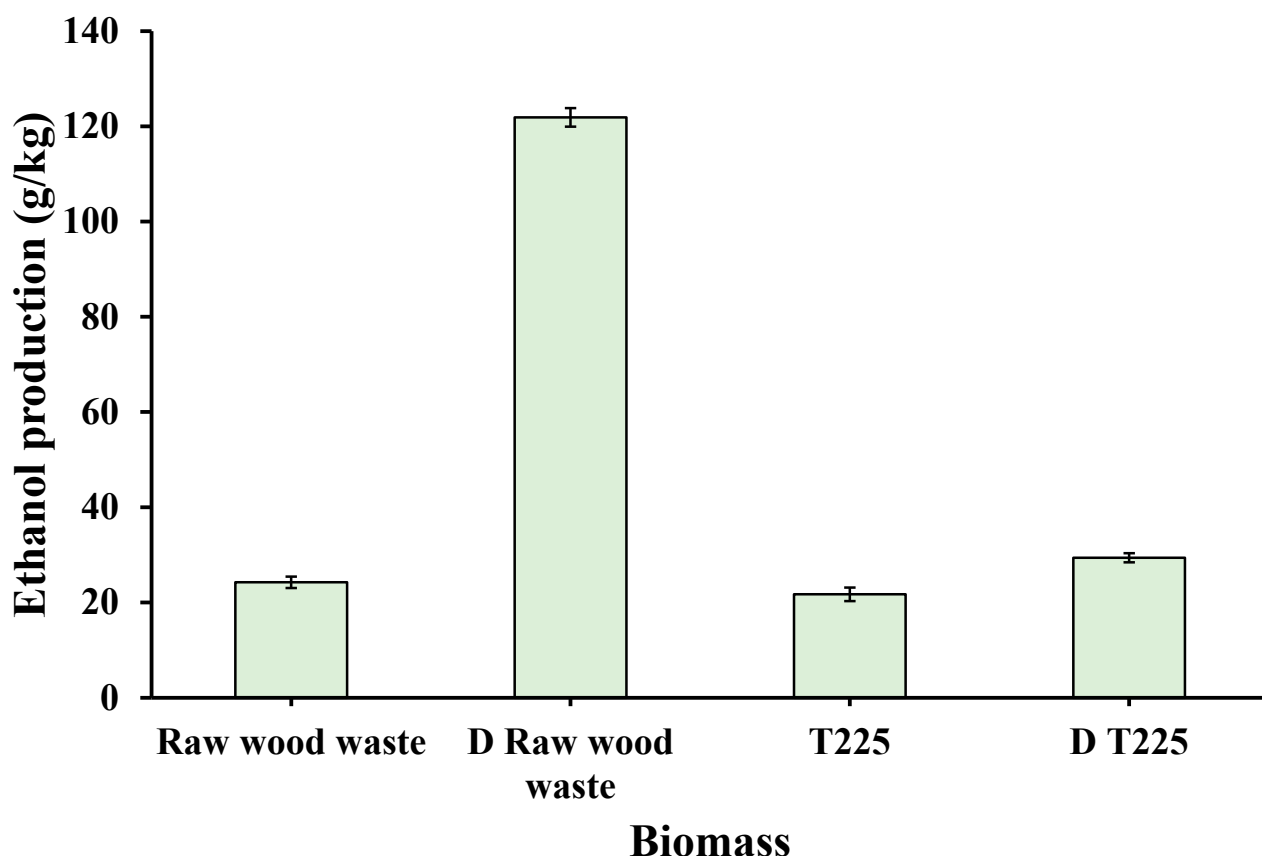


Figure 6. Bioethanol yield of raw and torrefied biomass.

After alkali treatment, bioethanol production from the RWW increased to >120 g/kg. This result was higher than that obtained by Bay et al. [36], who obtained an ethanol yield of 110 g/kg after alkali treatment at 93 °C with 8% sodium hydroxide at a ratio of 5% for 2 h. Compared to the T₂₂₅ biomass, the bioethanol production using DT₂₂₅ was significantly improved by approximately 35%, which reflects the importance of integrating torrefaction with alkaline pretreatment for exploring its feedstock potential for large-scale fermentation processes.

4. Conclusions

A three-step bioethanol production was performed to investigate the synergistic effects of torrefaction and alkali treatment, including pretreatment, hydrolysis, and fermentation, on increasing wood waste characteristics as a feedstock for liquid fuel production. Torrefaction at lower temperatures has the potential to be used as feedstock pretreatment for bioethanol production. Alkali pretreatment has a profound effect on TB, which improved the cellulose content and glucose production via enzymatic hydrolysis. Fur-

thermore, ethanol production from the integrated process was significantly increased to 26-fold over that of TB, indicating the efficiency of the integrated pretreatment. Based on these results, it is apparent that torrefaction at a lower temperature in conjunction with delignification can serve as a suitable integrated pretreatment process for ethanol fermentation, whereas torrefaction at a higher temperature is more suitable for combustion and gasification processes.

Author Contributions: Conceptualization, M.N.C., S.S. and T.K.; methodology, M.N.C. and S.S.; software, S.S. and M.N.C.; validation, M.N.C., S.S. and T.K.; formal analysis, M.N.C. and S.S.; investigation, M.N.C. and S.S.; resources, M.N.C. and S.S.; data curation, M.N.C. and S.S.; writing—original draft preparation, M.N.C. and S.S.; writing—review and editing, M.N.C., S.S. and T.K.; visualization, M.N.C., S.S. and T.K.; supervision, T.K.; project administration, T.K.; funding acquisition, T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by ERDF and the Baltic Research Programme project No. EEARESEARCH-173 “Novel biorefinery concepts for valorization of lignocellulosic residues (NoviCo)” under the EEA Grant of Iceland, Liechtenstein and Norway (Agreement No. EEZ/BPP/VIAA/2021/7) and Estonian Research Council via project RESTA5. M.N.C acknowledges the support from the European Regional Development Fund-DoraPlus-Estonia.

Data Availability Statement: The data presented in this study are available in the article.

Conflicts of Interest: The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

References

- Hasanov, I.; Shanmugam, S.; Kikas, T. Extraction and isolation of lignin from ash tree (*Fraxinus exselsior*) with protic ionic liquids (PILs). *Chemosphere* **2022**, *290*, 133297. [\[CrossRef\]](#)
- Ge, S.; Yek, P.N.Y.; Cheng, Y.W.; Xia, C.; Mahari, W.A.W.; Liew, R.K.; Peng, W.; Yuan, T.-Q.; Tabatabaei, M.; Aghbashlo, M.; et al. Progress in microwave pyrolysis conversion of agricultural waste to value-added biofuels: A batch to continuous approach. *Renew. Sustain. Energy Rev.* **2021**, *135*, 110148. [\[CrossRef\]](#)
- Nunes, L.; Causer, T.; Ciolkosz, D. Biomass for energy: A review on supply chain management models. *Renew. Sustain. Energy Rev.* **2020**, *120*, 109658. [\[CrossRef\]](#)
- Abdulyekeen, K.A.; Umar, A.A.; Patah, M.F.A.; Daud, W.M.A.W. Torrefaction of biomass: Production of enhanced solid biofuel from municipal solid waste and other types of biomass. *Renew. Sustain. Energy Rev.* **2021**, *150*, 111436. [\[CrossRef\]](#)
- Adeleke, A.A.; Odusote, J.K.; Ikubanni, P.P.; Lasode, O.A.; Malathi, M.; Paswan, D. Essential basics on biomass torrefaction, densification and utilization. *Int. J. Energy Res.* **2021**, *45*, 1375–1395. [\[CrossRef\]](#)
- Xin, S.; Huang, F.; Liu, X.; Mi, T.; Xu, Q. Torrefaction of herbal medicine wastes: Characterization of the physicochemical properties and combustion behaviors. *Bioresour. Technol.* **2019**, *287*, 121408. [\[CrossRef\]](#)
- Sarker, T.R.; Azargohar, R.; Dalai, A.K.; Meda, V. Enhancement of fuel and physicochemical properties of canola residues via microwave torrefaction. *Energy Rep.* **2021**, *7*, 6338–6353. [\[CrossRef\]](#)
- Chiaramonti, D.; Rizzo, A.M.; Prussi, M.; Tedeschi, S.; Zimbardi, F.; Braccio, G.; Viola, E.; Pardelli, P.T. 2nd generation lignocellulosic bioethanol: Is torrefaction a possible approach to biomass pretreatment? *Biomass Convers. Biorefinery* **2011**, *1*, 9–15. [\[CrossRef\]](#)
- Chaluvadi, S.; Ujjwal, A.; Singh, R. Effect of torrefaction prior to biomass size reduction on ethanol production. *Waste Biomass Valorization* **2019**, *10*, 3567–3577. [\[CrossRef\]](#)
- Sheikh, M.I.; Kim, C.-H.; Park, H.-J.; Kim, S.-H.; Kim, G.-C.; Lee, J.-Y.; Sim, S.-W.; Kim, J.W. Effect of torrefaction for the pretreatment of rice straw for ethanol production. *J. Sci. Food Agric.* **2013**, *93*, 3198–3204. [\[CrossRef\]](#)
- Ong, H.C.; Yu, K.L.; Chen, W.-H.; Pillejera, M.K.; Bi, X.; Tran, K.-Q.; Pétrissans, A.; Pétrissans, M. Variation of lignocellulosic biomass structure from torrefaction: A critical review. *Renew. Sustain. Energy Rev.* **2021**, *152*, 111698. [\[CrossRef\]](#)
- Shinde, S.D.; Meng, X.; Kumar, R.; Ragauskas, A.J. Recent advances in understanding the pseudo-lignin formation in a lignocellulosic biorefinery. *Green Chem.* **2018**, *20*, 2192–2205. [\[CrossRef\]](#)
- Normark, M.; Pommer, L.; Gräsvik, J.; Hedenström, M.; Gorzsás, A.; Winestrand, S.; Jönsson, L.J. Biochemical Conversion of Torrefied Norway Spruce After Pretreatment with Acid or Ionic Liquid. *BioEnergy Res.* **2016**, *9*, 355–368. [\[CrossRef\]](#)
- Wen, J.-L.; Sun, S.-L.; Yuan, T.-Q.; Xu, F.; Sun, R.-C. Understanding the chemical and structural transformations of lignin macromolecule during torrefaction. *Appl. Energy* **2014**, *121*, 1–9. [\[CrossRef\]](#)
- Yang, Q.; Pan, X. Correlation between lignin physicochemical properties and inhibition to enzymatic hydrolysis of cellulose. *Biotechnol. Bioeng.* **2016**, *113*, 1213–1224. [\[CrossRef\]](#)
- Cahyanti, M.N.; Doddapaneni, T.R.K.C.; Madisoo, M.; Pärn, L.; Virro, I.; Kikas, T. Torrefaction of Agricultural and Wood Waste: Comparative Analysis of Selected Fuel Characteristics. *Energies* **2021**, *14*, 2774. [\[CrossRef\]](#)

17. Rooni, V.; Sjulander, N.; Cristobal-Sarramian, A.; Raud, M.; Rocha-Meneses, L.; Kikas, T. The efficiency of nitrogen explosion pretreatment on common aspen–*Populus tremula*: N₂–VS steam explosion. *Energy* **2021**, *220*, 119741. [\[CrossRef\]](#)
18. CEN/TS 14775; Solid Biofuels—Method for the Determination of Ash Content. European Committee for Standardization: Brussels, Belgium, 2004.
19. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. Determination of sugars, byproducts, and degradation products in liquid fraction process samples. *Gold. Natl. Renew. Energy Lab.* **2006**, *11*, 65–71.
20. Sjulander, N.; Kikas, T. Two-Step Pretreatment of Lignocellulosic Biomass for High-Sugar Recovery from the Structural Plant Polymers Cellulose and Hemicellulose. *Energies* **2022**, *15*, 8898. [\[CrossRef\]](#)
21. Yuan, Y.; Jiang, B.; Chen, H.; Wu, W.; Wu, S.; Jin, Y.; Xiao, H. Recent advances in understanding the effects of lignin structural characteristics on enzymatic hydrolysis. *Biotechnol. Biofuels* **2021**, *14*, 1–20.
22. Lee, K.H.; Lee, S.K.; Lee, J.; Kim, S.; Kim, S.W.; Park, C.; Yoo, H.Y. Energy-efficient glucose recovery from chestnut shell by optimization of NaOH pretreatment at room temperature and application to bioethanol production. *Environ. Res.* **2022**, *208*, 112710. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Yan, X.; Cheng, J.-R.; Wang, Y.-T.; Zhu, M.-J. Enhanced lignin removal and enzymolysis efficiency of grass waste by hydrogen peroxide synergized dilute alkali pretreatment. *Bioresour. Technol.* **2020**, *301*, 122756. [\[CrossRef\]](#)
24. Woiciechowski, A.L.; Neto, C.J.D.; de Souza Vandenbergh, L.P.; de Carvalho Neto, D.P.; Sydney, A.C.N.; Letti, L.A.J.; Karp, S.G.; Torres, L.A.Z.; Soccol, C.R. Lignocellulosic biomass: Acid and alkaline pretreatments and their effects on biomass recalcitrance—Conventional processing and recent advances. *Bioresour. Technol.* **2020**, *304*, 122848. [\[CrossRef\]](#)
25. Morales-Martínez, J.L.; Aguilar-Uscanga, M.G.; Bolaños-Reynoso, E.; López-Zamora, L. Optimization of Chemical Pretreatments Using Response Surface Methodology for Second-Generation Ethanol Production from Coffee Husk Waste. *BioEnergy Res.* **2021**, *14*, 815–827. [\[CrossRef\]](#)
26. Corderi, S.; Renders, T.; Servaes, K.; Vanbroekhoven, K.; De Roo, T.; Elst, K. Strategies for the Removal of Polysaccharides from Biorefinery Lignins: Process Optimization and Techno Economic Evaluation. *Molecules* **2021**, *26*, 3324. [\[CrossRef\]](#)
27. Tripathi, J.; Richard, T.L.; Memis, B.; Demirci, A.; Ciolkosz, D. Interactions of Torrefaction and Alkaline Pretreatment with Respect to Glucose Yield of Hydrolyzed Wheat Straw. *Biomass* **2022**, *2*, 264–278. [\[CrossRef\]](#)
28. Yang, H.; Yan, R.; Chen, H.; Lee, D.H.; Zheng, C. Characteristics of hemicellulose, cellulose and lignin pyrolysis. *Fuel* **2007**, *86*, 1781–1788. [\[CrossRef\]](#)
29. Zhang, Z. Waste pretreatment technologies for hydrogen production. In *Waste to Renewable Biohydrogen*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 109–122.
30. Gagić, T.; Perva-Uzunalić, A.; Knez, Ž.; Škerget, M. Hydrothermal Degradation of Cellulose at Temperature from 200 to 300 °C. *Ind. Eng. Chem. Res.* **2018**, *57*, 6576–6584. [\[CrossRef\]](#)
31. Manokhoon, P.; Rangseesuriyachai, T. Effect of two-stage sodium hydroxide pretreatment on the composition and structure of Napier grass (*Pennisetum purpureum*). *Int. J. Green Energy* **2020**, *17*, 864–871. [\[CrossRef\]](#)
32. Kathirselvam, M.; Kumaravel, A.; Arthanarieswaran, V.; Saravanakumar, S. Characterization of cellulose fibers in *Thespesia populnea* barks: Influence of alkali treatment. *Carbohydr. Polym.* **2019**, *217*, 178–189. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Verma, S.; Midha, V.K.; Choudhary, A.K. Optimization of Parameters for Alkali Pretreatment on Coir Fiber for Biomass Production Using TOPSIS. *J. Nat. Fibers* **2022**, *19*, 3038–3050. [\[CrossRef\]](#)
34. Oh, S.; Park, D.H.; Lee, S.M.; Ahn, B.J.; Ahn, S.H.; Yang, I. Effect of Torrefaction Temperature on Lignin Distribution of *Larix kaempferi* C. and *Liriodendron tulipifera* L. Cubes and the Impact of Binder on Durability of Pellets Fabricated with the Torrefied Cubes. *Environ. Prog. Sustain. Energy* **2019**, *38*, 13190. [\[CrossRef\]](#)
35. Noori, M.S.; Karimi, K. Chemical and structural analysis of alkali pretreated pinewood for efficient ethanol production. *RSC Adv.* **2016**, *6*, 65683–65690. [\[CrossRef\]](#)
36. Bay, M.S.; Karimi, K.; Esfahany, M.N.; Kumar, R. Structural modification of pine and poplar wood by alkali pretreatment to improve ethanol production. *Ind. Crop. Prod.* **2020**, *152*, 112506. [\[CrossRef\]](#)
37. Shi, L.; Hu, Z.; Li, X.; Li, S.; Yi, L.; Wang, X.; Hu, H.; Luo, G.; Yao, H. Gas-pressurized torrefaction of lignocellulosic solid wastes: Low-temperature deoxygenation and chemical structure evolution mechanisms. *Bioresour. Technol.* **2023**, *385*, 129414. [\[CrossRef\]](#) [\[PubMed\]](#)
38. He, N.; Chen, M.; Qiu, Z.; Fang, C.; Lidén, G.; Liu, X.; Zhang, B.; Bao, J. Simultaneous and rate-coordinated conversion of lignocellulose derived glucose, xylose, arabinose, mannose, and galactose into D-lactic acid production facilitates D-lactide synthesis. *Bioresour. Technol.* **2023**, *377*, 128950. [\[CrossRef\]](#)
39. Hu, Z.; Wang, Y.; Liu, J.; Li, Y.; Wang, Y.; Huang, J.; Ai, Y.; Chen, P.; He, Y.; Aftab, M.N.; et al. Integrated NIRS and QTL assays reveal minor mannose and galactose as contrast lignocellulose factors for biomass enzymatic saccharification in rice. *Biotechnol. Biofuels* **2021**, *14*, 144. [\[CrossRef\]](#)
40. Auxenfans, T.; Crônier, D.; Chabbert, B.; Paës, G. Understanding the structural and chemical changes of plant biomass following steam explosion pretreatment. *Biotechnol. Biofuels* **2017**, *10*, 36. [\[CrossRef\]](#)
41. Bhatia, R.; Lad, J.B.; Bosch, M.; Bryant, D.N.; Leak, D.; Hallett, J.P.; Franco, T.T.; Gallagher, J.A. Production of oligosaccharides and biofuels from *Miscanthus* using combinatorial steam explosion and ionic liquid pretreatment. *Bioresour. Technol.* **2021**, *323*, 124625. [\[CrossRef\]](#)

42. Lin, Y.-Y.; Chen, W.-H.; Colin, B.; Pétrissans, A.; Quirino, R.L.; Pétrissans, M. Thermodegradation characterization of hardwoods and softwoods in torrefaction and transition zone between torrefaction and pyrolysis. *Fuel* **2022**, *310*, 122281. [\[CrossRef\]](#)
43. Li, Y.; Yan, C.; Chen, Y.; Han, X.; Shao, Z.; Qi, H.; Li, X.; Nishiyama, Y.; Hu, T.; Chen, P. The major role of London dispersion interaction in the assembly of cellulose, chitin, and chitosan. *Cellulose* **2023**, *30*, 8127–8138. [\[CrossRef\]](#)
44. Haldar, D.; Dey, P.; Patel, A.K.; Dong, C.-D.; Singhania, R.R. A Critical Review on the Effect of Lignin Redeposition on Biomass in Controlling the Process of Enzymatic Hydrolysis. *BioEnergy Res.* **2022**, *15*, 863–874. [\[CrossRef\]](#)
45. Kim, S. Enhancing Bioethanol Productivity Using Alkali-Pretreated Empty Palm Fruit Bunch Fiber Hydrolysate. *BioMed Res. Int.* **2018**, *2018*, 5272935. [\[CrossRef\]](#)
46. Toquero, C.; Bolado, S. Effect of four pretreatments on enzymatic hydrolysis and ethanol fermentation of wheat straw. Influence of inhibitors and washing. *Bioresour. Technol.* **2014**, *157*, 68–76. [\[CrossRef\]](#)
47. Ellison, C.; Garcia-Perez, M.; Mullen, C.A.; Yadav, M.P. Thermochemical behavior of alkali pretreated biomass—A thermogravimetric and Py-GC/FID study. *Sustain. Energy Fuels*. **2023**, *7*, 3306–3315. [\[CrossRef\]](#)
48. Salehian, P.; Karimi, K. Alkali Pretreatment for Improvement of Biogas and Ethanol Production from Different Waste Parts of Pine Tree. *Ind. Eng. Chem. Res.* **2013**, *52*, 972–978. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.