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Research Article

Microwave-assisted potassium hydroxide pretreatment to enhance enzymatic hydrolysis of rubberwood (*hevea brasiliensis*) sawdust

L.A. Wati[1], S. Selpiana[1,2] and N. Novia[1,2,3]*

ABSTRACT: Rubberwood sawdust, a lignocellulosic resource derived from industrial and agricultural waste, has the potential for conversion into biofuel. Rubberwood sawdust underwent pretreatment to enhance the accessibility of cellulose to enzymes. The pretreated rubberwood sawdust was subsequently mixed with potassium hydroxide (KOH) solution at 1% and 2% (w/v) concentrations. Each mixture was heated conventionally for 30 min, followed by microwave penetration for 5, 10, 15, 20, and 25 min that operated at 360 Hz, with a power level of 10% (input microwave power 150 Watts). Enzymatic hydrolysis was conducted on the pretreated samples with enhanced cellulose yields for 1 h at 50°C. The result indicated that lignin content decreased from 29.83% (w/w) to 20.00% (w/w) and 17.36% (w/w) for 1% and 2% (w/v) KOH 25 min microwave penetration, respectively. The highest cellulose 43.73% (w/w), was obtained by 2% (w/v) KOH 25 min microwave duration. Samples were hydrolyzed for 1 h, 2% (w/v) KOH 25 min microwave exposure reached 0.027 g/L fermentable sugars. This method affected to lignin degradation, enhanced cellulose content to achieve higher sugar. The higher concentration of KOH resulted in significant lignin degradation. The microwave allowed for handling heat faster, saves energy and time, and creates less pretreatment waste.

Keywords: *Rubberwood sawdust, Potassium hydroxide, Sugar, Microwave irradiation, Lignin removal*

1. INTRODUCTION

Renewable and environmentally sustainable bioenergy is still become the interest of the expert. It can be derived from biomass materials that produce low greenhouse gas emissions and do not interfere with food requirements, including forestry residues like wood chips, sawdust, and tree bark [1]. Additionally, using lignocellulosic biomass is linked to cheaper fuels and chemicals and less reliance on fossil fuels. In addition to lowering landfill waste, biomass generation gives manufacturers a new revenue stream. Rubber plantations are prevalent in several regions globally, with over 75 percent situated in Indonesia, Thailand, and Malaysia [2]. In 2023, South Sumatra Province boasts the most significant extent of smallholder rubber plantations in Indonesia, covering 743.88 thousand hectares, which is responsible for 26.1% of the national total for such plantations [3]. Various sectors utilize rubberwood materials, such as the fibreboard industry and Pulp and Paper manufacturing sectors [4]. These industries left the residue like sawdust. Additionally, rubberwood sawdust presents a promising opportunity for the production of fermentable sugar, which can be transformed into second-generation bioethanol [8]. The rubberwood sawdust comprises 77% holocellulose [5]. Cellulose hydrolysis is an important process for producing sugar, but the use of enzymatic hydrolysis has limited effectiveness. By breaking the cross-links in lignocellulose, the bond between lignin and hemicellulose is released, thereby increasing enzyme access during hydrolysis [6]. Lignocellulosic biomass is primarily composed of lignin, cellulose, hemicellulose, and various other components [7]. Lignin is a complex polymer characterized by its inherent rigidity and resistance to degradation. Pretreatment is

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necessary as lignin is the primary inhibitor in the cellulose hydrolysis process. Several studies have previously examined the pretreatment of rubberwood sawdust using acids (H_2SO_4) [8], which has potential to generate hydroxy methyl furfural, and risk of corrosion [9]. Rubberwood sawdust undergoes alkali pretreatment, such as NaOH, before hydrolysis to yield sugar [10]. Nonetheless, the utilization of NaOH compounds still necessitates the management of sodium waste. The traditional approach to pretreatment involving high temperatures and pressures faces constraints due to significant energy consumption, highlighting the necessity for alternative heating methods that can also enhance efficiency. The use of microwave technology in the delignification of lignocellulose has several benefits, such as efficient and consistent heating that may expedite chemical processes and improve process efficacy [11]. Moreover, microwave treatment may alter the physical structure of lignocellulose, enhancing the accessibility of the cellulose fraction for following operations and facilitating a decrease in chemical use [12]. Consequently, this technology is seen as more ecologically sustainable and has the potential to serve as a viable alternative to traditional methods. This study investigates the conversion of rubber wood sawdust into sugar through a combination of potassium hydroxide and microwave pretreatment, followed by enzymatic hydrolysis. This approach seeks to enhance production, reduce costs, and explore the potential of rubber wood residues. By integrating the revolutionary microwave-assisted potassium hydroxide pretreatment to develop an efficient technique for producing reducing sugars, characterised by rapid and low-temperature heating, using more ecologically sustainable and cost-effective method. The findings of the lignin degradation study are examined to provide direction for optimisation by identifying variables and pretreatment operational parameters to get maximal reducing sugars. Consequently, it signifies considerable advancement in renewable energy and waste valorisation.

2. METHODOLOGY

Rubberwood (Hevea brasisiliensis) sawdust was sourced from fibreboard industry Inderalaya, South Sumatra Province, Indonesia. potassium hydroxide (KOH) from Seoul, South Korea, at Unid.co Ltd. Additionally, Aspergillus niger was obtained from the biochemistry laboratory of Sriwijaya University. Merck Indonesia supplied all the residual chemicals. The Sharp R-728 microwave device operates at 360 Hz and has a power setting of 10%, which configures the input microwave power to 150 watts.

2.1. Raw material preparation and microwave assisted potassium hydroxide pretreatment. Raw Material Preparation : Rubberwood sawdust was air-dried for 1 week, subsequently sieved to achieve a 30 mesh particle size. Then, it was dried in an oven at 50°C for 3 x 8 h until a steady mass was achieved and stored at room temperature. The samples were analyzed for lignin, cellulose, and hemicellulose components utilizing the Chesson and Sem-EDX method. Microwave Assisted Potassium Hydroxide Pretreatment: The prepared rubberwood sawdust (Hevea brasiliensis) was placed into an flask, followed by the addition of a KOH solution at concentrations of 1% and 2% (w/v), which was mixed until a homogeneous mixture was achieved. The proportion of biomass to KOH solution is 1:10 (100 g of biomass corresponds to 1000 mL of distilled water). Subsequently, the mixture was subjected to a hotplate stirrer at a temperature range of 70-80°C for 30 min, followed by microwave treatment with changing time of 5, 10, 15, 20, and 25 min. Subsequently, the mixture underwent neutralization with distilled water, followed by filtration and drying in an oven at a temperature range of 100-110°C. Samples were stored and subsequently examined utilizing the Chesson method and SEM-EDX.

2.2. Producing cellulase enzymes from rubberwood sawdust. The inoculum formulation involved the preparation of 100 mL of liquid media, which included 0.25% (w/v) $(NH_4)_2SO_4$, 12.5% (w/v) sucrose, and 0.2% (w/v) KH_2PO_4. pH of the liquid was set to 3 using citric acid. Equipment and liquid media underwent sterilization through an autoclave for 15 min at 121°C, then cooled to room temperature. Aspergillus niger from PDA media were aseptically transferred to liquid media and incubated 24 h at 30°C in an incubator. The preparation of enzyme growth media involved 20 g rubberwood sawdust, supplied nutrients of 0.005 g $MgSO_4 \cdot 7H_2O$, 0.03 g urea, and 0.0023 g KH_2PO_4. It was combined with 80 mL of demineralized water, adjusted pH 5 [13]. Subsequently, 10 mL of formulated inoculant was introduced into the enzyme growth medium and kept at room temperature 96 h fermentation. The fermentation liquid was added demineralized water 100 mL in a rotary shaker 1 h at 150 rpm. The sample centrifuged at 4000 rpm 30 min at 4°C. The crude enzyme obtained as a liquid phase.

2.3. Enzymatic Hydrolysis. All equipment underwent sterilization. The proportion of 20 g rubberwood sawdust to deionized water utilized was 1:10 (w/v) at pH 5, adapted with sodium citrate buffer. The samples performed to heat at 50°C, then stirred at 200 rpm and experienced hydrolysis for 1 h. Following hydrolysis, the filtrate was cooled, and the fermentable sugar concentration was assessed utilizing DNS fluid and a UV-Vis spectrophotometer [14]. The enzymatic hydrolysis is illustrated in Figure 1.

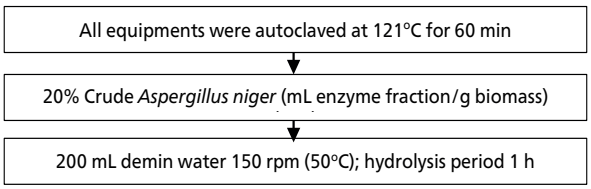

Figure 1. Enzymatic hydrolysis scheme.

2.4. Rubberwood Sawdust Composition Analysis. The Chesson method [15] conducted to analyze the components of rubberwood sawdust. 1 g (a) of the dried solid underwent reflux for 1 h at 100°C with 150 mL of deionized water, followed by filtration and drying in an oven, after which it was weighed (b). The sample underwent reflux for 1 h at 100°C with 150 mL of 1N H_2SO_4, followed by filtration, neutralization, drying, and weighing (c). Additionally, the sample underwent a soaking process 72% H_2SO_4 10 mL for 4 h at room temperature, which was followed by 1 h reflux period with 1 N H_2SO_4 150 mL at 100°C. The sample underwent filtration, neutralization, drying, and weighing (d). The sample was placed the furnace at 550°C (e). The equation below was employed to determine the quantities of lignin, cellulose, and hemicellulose.

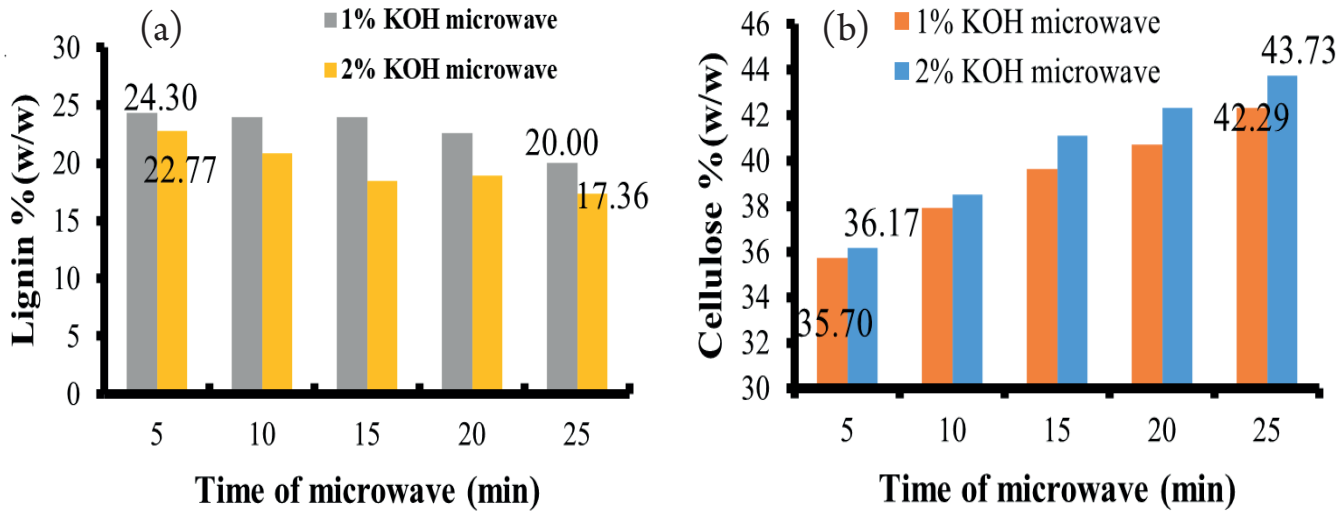

Figure 2. Evaluation of microwave penetration time concerning (a) lignin and (b) cellulose content of pretreated rubberwood sawdust.

Cellulose	= (c-d)/a .100%	(1)
Hemicellulose	= (b-c)/a. 100%	(2)
Lignin	= (d-e)/a. 100%	(3)

2.5. Morphological Sample Analysis and Reducing Sugar Analysis. Morphological Sample Analysis: Axia ChemiSem SEM-EDX performed to illustrate the elemental composition and surface morphology of rubberwood sawdust before and after treatment with magnification scale 50 μm. Reducing Sugar Analysis: a mL of hydrolysed solution was injected, then 3 mL DNS reagent was combined and heated for 5 min at 100°C. 1 mL of hydrolyzed solution was injected to a tube, 3 mL of DNS reagent inserted and combined, then 5 min heated at 100°C in a water bath. The sample was placed into a cuvette. UV absorbance was measured using UV-1800 UV-Vis Spectrophotometer at 540 nm under 3 repetitions [16].

3. RESULT AND DISCUSSION

3.1. Delignification. Figure 2(a) presented a comparison of the length of microwave exposure time for rubberwood sawdust that had undergone pretreatment with 1% (w/v) and 2% (w/v) KOH. Lignin levels decreased from 29.83% (w/w) to 20.00% (w/w) and 17.36% (w/w) for 1% (w/v) and 2% (w/v) KOH for 25 microwave exposure, respectively. The results indicated that KOH and microwave promoted lignin solubilization. Lignin concentration reduced from 29.83% (w/w) to 23.99% (w/w) and 18.44% (w/w) for 1% (w/v) and 2% (w/v) KOH after 15 min of microwave exposure. The adequate heating periods of 15 and 25 min, KOH sufficiently infiltrated the cell wall structure and efficiently disrupted the bonds (ester and ether linkages), effected the liberation of lignin from the lignocellulosic matrix. The microwave facilitated volumetric heating, directly affected water and other polar molecules, hence accelerated chemical processes [12]. The use of microwave heat enhanced the efficiency of the delignification process using KOH by facilitating the penetration of KOH and expediting the lignin breakdown reaction [17]. After 15 min, the majority of the readily removable lignin has already decomposed. A higher concentration of KOH resulted in significant lignin removal [18]. Figure 2(b) illustrated level of cellulose. The highest cellulose level obtained was 43.73% (w/w) after pretreatment by 2% (w/v) KOH for 25 min microwave. An alkaline solution functions as a chemical bond-breaking agent, disrupting the lignin structure within the amorphous crystal and partially separating hemicellulose. This alteration facilitated changes in structure and properties, enhanced solubility in water [19]. In this experiment, the further microwave pretreatment time released more cellulose from the lignin bond structure. Within a reasonable range, the microwave resulted in structural damage to the cell wall and accelerated chemical reactions, leading to the decomposition of lignin and releasing the free cellulose into the solution [20]. The biopolymers O-H bonds effectively absorbed microwaves, resulting in rapid heating and elevated pressure. Conversely, components with inadequate hydration or low polarity did not undergo expansion [17].

3.2. Scanning Electron Microscopy–Energy Disperse Xray (SEM-EDX Analysis). Shown by Figure 3(a) that rubberwood sawdust indicated a rigid and very regular surface structure that contained strong bonds between the initial fibre bundle and the noncellulosic portion [10]. The middle lamella exhibited a precise arrangement. As illustrated in Figure 3(b) and (c). The matrix of lignocellulose material was beginning to exhibit deterioration. The integrity of the middle lamella began to stretch as reported by Ali [5]. Numerous fissures were observable in the cellular structures situated between the two fibres, leading to an increase in surface area. As the concentration of alkali increased was described by Figure 3(c). Pretreated rubberwood resulted in a flat morphology due to deconstruction of the rigid structure of rubberwood caused by lignin removal process, as previous analysis of wasted rice husk indicated comparable trends [21]. Illustrated

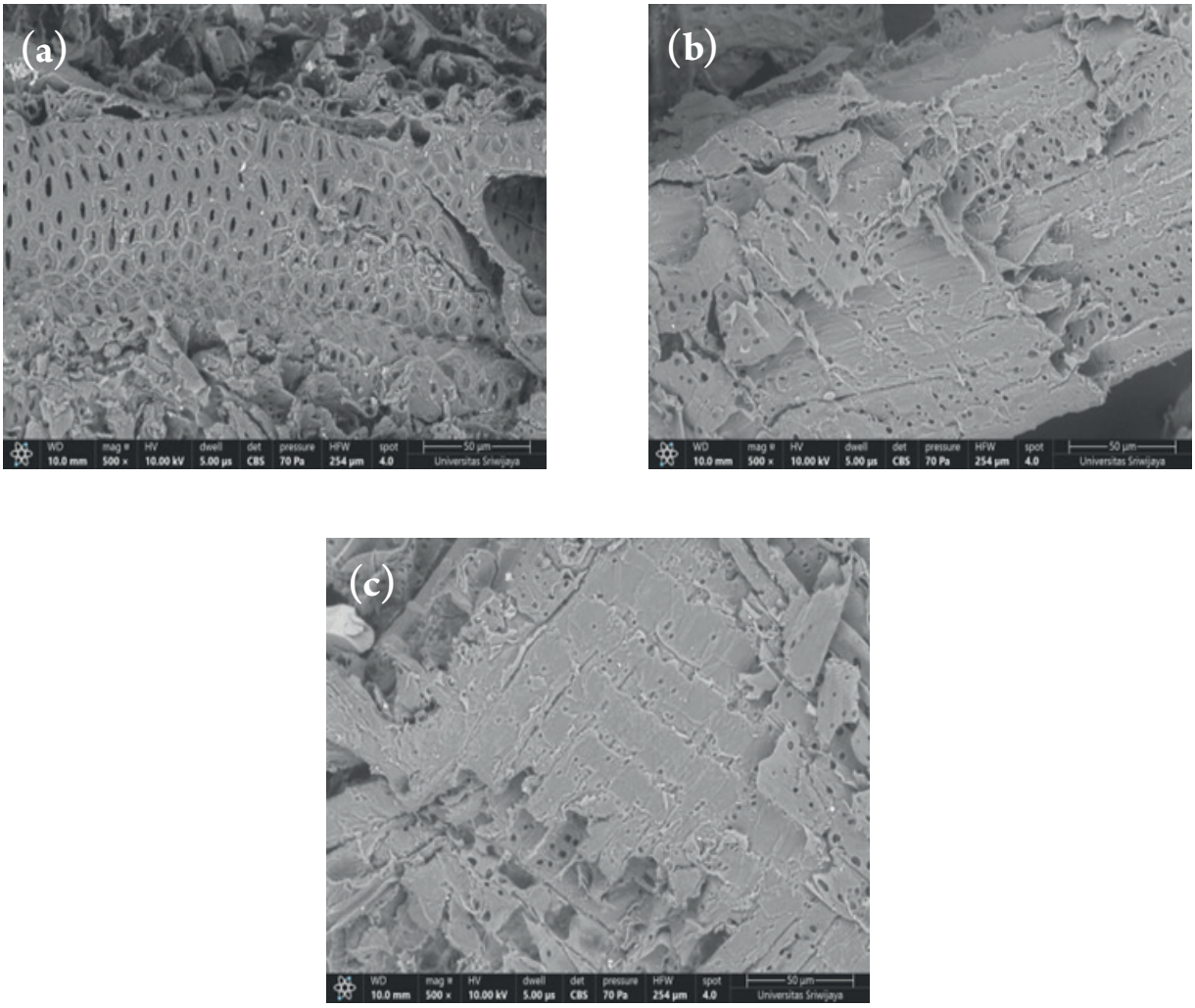

Figure 3. Morphology of rubberwood sawdust (Hevea brasiliensis): (a) Untreated, (b) Pretreated with 1% (w/v) KOH microwave 25 min, (c) Pretreated with 2% (w/v) KOH microwave 25 min.

in Table 1 carbon content increased from untreated 36.9% (w/w) to 40.9% (w/w) and 41.2% (w/w), 1% (w/v), and 2% (w/v) KOH for 25 min microwave period, respectively. The detected rise in carbon suggested that the pretreatment phase improved the cellulose component, while decreasing in the magnesium [14]. The presence of Mg in the raw material was observed in small quantities, which diminished following the delignification process, suggesting that delignification of rubberwood sawdust resulted in the elimination of extractives, as reported by Paixão [22]. The decrease in oxygen was insignificant from untreated 56.6% (w/w) to 56.4% (w/w) and 46.0% (w/w), 1% (w/v), and 2% (w/v) KOH for 25 min microwave penetration, respectively. Due to the reduction in ash content, the total amounts of O and Mg decreased when pretreatment was carried out [23].

3.3. Enzymatic Hydrolysis of to Produce Fermentable Sugar. The enzymatic hydrolysis experiment was performed in triplicate following the methodology outlined by Efrinalia [14]. In this work, the longest microwave exposure duration 25 min KOH 2% (w/v) pretreated rubberwood sawdust was enzymatically hydrolysed by crude cellulase enzyme for 1 h to produce fermentable sugar. The cellulase enzyme derived from Aspergillus niger was appropriate for the enzymatic hydrolysis of lignocellulosic biomass owing to its elevated activity and stability [24]. The microwave irradiation resulted in fast heating and generated dielectric heat via direct contact among rubberwood particles, led to the disintegration of the rubberwood sawdust matrix, then caused easier access of cellulose enzymes. From Table 2 showed the achieved fermentable sugar from this work which compared to others works.

Refer to Table 2, the study revealed that the yield of reducing sugars was lower to that of prior research due to the hydrolysis incubation period being limited to only 1 h, much less than other approaches that extend up to 72 hours [8]. The raw material, Hevea brasiliensis sawdust, has distinct cellulose characteristics in contrast to the rubberwood waste or rubberwood sawdust used in earlier research. Commercial enzymes like Cellic®CTec2 or Trichoderma reesei shown more effectiveness in prior investigations [27]. Parameters like pH, temperature, enzyme concentration, substrate-enzyme ratio, and sterility control influence the outcomes of hydrolysis. Suboptimal circumstances may result in a significantly reduced sugar production [28].

Table 1. Component Comparison of Untreated and Pretreated Rubberwood Sawdust.

Element	Untreated, % (w/w)	1% (w/v) KOH 25 min mic, % (w/w)	2% (w/v) KOH 25 min mic, % (w/w)
C	36.9	40.9	41.2
O	56.5	56.4	46.0
Mg	0.6	0.3	0.4
Ca	0.7	1.2	1.2

Table 2. Comparison of pretreated rubberwood to produce sugar.

Raw material	Method	Sugar	Ref
Rubberwood waste	Pretreatment: Organosolv 210°C; 30 min	55 g/L	[25]
Rubberwood waste	Pretreatment: Steam explosion 20 bar; 10 min. Hydrolysis: enzyme Cellic®CTec2 30 FPU/g	118 g/kg	[26]
Rubberwood sawdust	Pretreatment: Dilute H_2SO_4 and NaOH Hydrolysis: Trichoderma reesei 72 h	46.13 g/L	[8]
Hevea brasiliensis sawdust	Pretreatment: 2% (w/v) KOH - 25 min microwave. Hydrolysis: Aspergillus niger 1 h	0.027 g/L	This work

4. CONCLUSION

This research characterized untreated and pretreated rubberwood sawdust. Potassium hydroxide microwave-assisted pretreatment affected lignin content, enhanced the cellulose content, and facilitated the enzymatic hydrolysis of cellulose to achieve sugar yield as a material for bioethanol, and varied concentrations of potassium hydroxide were comparable to the cellulose, lignin component. The artwork obtained using SEM demonstrated that the biomass structure deformed and delignified as the intensity of the pretreatment increased. The result showed that the highest cellulose level, which was obtained at 43.73% (w/w) after pretreated by 2% (w/v) KOH for 25 min microwave, achieved the fermentable sugar of 0.027 g/L when enzymatically hydrolyzed for 1 h. The microwave assists in managing heat easier, saves energy and time, and produces less pretreatment waste

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CREDIT AUTHOR STATEMENT

Luki Anugrah Wati: Conceptualization, Investigation, Formal analysis, Writing original draft, Methodology. **Selpiana Selpiana**: Data curation, Formal analysis, Supervision, Writing-Reviewing, and Editing. **Novia Novia**: Conceptualization, Resources, Supervision, Writing-Reviewing, and Editing.

DECLARATIONS

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

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