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Effect of microwave and ultrasonic irradiation on the enzymatic hydrolysis of water hyacinth biomass in the presence of surfactants

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Abstract

This paper presents the effect of microwave and ultrasound irradiation on the enzymatic hydrolysis of water hyacinth biomass in the presence of surfactants. Prior to hydrolyzing, the water hyacinth was treated utilizing alkali with and without microwave assistance. It was revealed that the microwave improved the removal of lignin and hemicellulose. The treated water hyacinth biomass was also characterized using Scanning Electron Microscope (SEM), X-ray Diffraction (XRD), and Fourier-Transform Infrared (FT-IR) Spectroscopy. The effect of surfactant and the assistance of microwave and ultrasound were comprehensively studied. Some parameters varied, including stirring speed, surfactant type, concentration, and reaction time. The results indicated that microwave and ultrasound could enhance the reaction rate. Tween 80 here could improve conventional, microwave-assisted, and ultrasound-assisted hydrolysis of water hyacinth biomass. It was found that the ultrasound-assisted hydrolysis was better than that of others. The results of this research can be used as the groundwork for further developing the lignocellulosic biomass hydrolysis process, especially in an advanced enzymatic hydrolysis process.

Keywords: Surfactant Tween 80; hydrolysis; water hyacinth; microwave; ultrasonic

1. Introduction

Increasing consumption and demand for petroleum and other fuel sources have emerged a number of environmental issues such as climate change and global warming. The depletion of fossil-based energy sources and the continuous fluctuations in petroleum prices have resulted in more efforts to produce biofuels from renewable resources [1]. Biofuel from lignocellulosic biomass is a potential preference to substitute fossil fuels [2]. Lignocellulosic biomass is the foremost plentiful feedstock in the world to produce bioenergy and biofuels through hydrolysis and fermentation [3]. Its sources include agricultural wastes such as rice and wheat straws, corn stover, cornstalks, and sugarcane bagasse. Alternatively, Mezule et al. [4] utilized the fermentable carbohydrate from natural grasslands biomass as a source of biofuel production. In this study, water hyacinth was used as the lignocellulosic biomass feedstock.

Water hyacinth is an invasive aquatic plant with a high growth rate, so it is considered a weed with negative impacts on ecosystem. It can form a mat of water hyacinth blocking the sunlight and oxygen from entering the waters and reducing water quality. Therefore, it is deemed necessary to control the growth of water hyacinth. One method to control it is by harvesting the plant and using it as a source of cellulose. Water hyacinth mostly contains hemicellulose, cellulose, and lignin, each of which is generally 48%, 18%, and 3.5%, respectively [5]. The abundant availability of this plant has made it a qualified feedstock for biofuels production.

The lignocellulosic biomass conversion to biofuels can be conducted through thermo-chemical conversion [6,7]. Through this process, lignocellulosic biomass can be directly converted into hydrocarbon fuels. This process, however, requires a high energy level and is generally conducted at high temperature (~800°C) [6]. Another method of lignocellulosic biomass conversion is through fermentation, producing bioethanol [8].

Hydrolysis is a main process in biofuels synthesis from lignocellulosic biomass. It converts biomass to reduce sugars that are further fermented to desired biofuels, such as bioethanol, or biobutanol using appropriate microorganisms. Compared to other hydrolysis methods, enzymatic hydrolysis is widely applied due to the lack of corrosion, its mild reaction conditions, and favorable environmental profile [9]. However, the complex structure of lignocellulose causes the low accessibility of enzymes to cellulose [10,11]. Figure1 overall depicts the reaction mechanism of enzymatic hydrolysis of cellulose.

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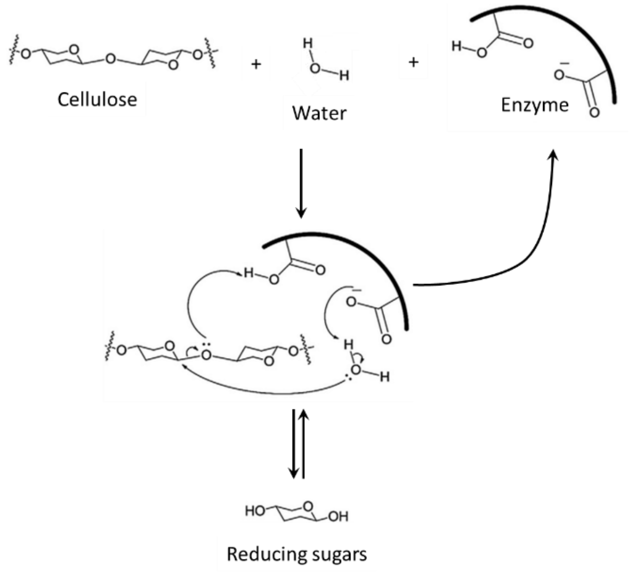

Fig. 1. Reaction mechanism of enzymatic hydrolysis of cellulose to reducing sugars.

Many researchers have attempted to enhance the enzymatic hydrolysis of lignocellulose [12–15]. The pretreatment of lignocellulosic biomass is regarded as a needful process in the hydrolysis of cellulose. Crystalline cellulose is interrelated with hemicellulose and lignin, forming a lignocellulose matrix. The conformation and arrangement of lignocellulosic molecules have made them highly resistant to any chemical and enzymatic hydrolysis. Pretreatment can break the structure of lignocellulose, remove unwanted components, reduce cellulose crystallinity, and increase porosity, making cellulose more accessible for hydrolysis process [16]. The purpose of pretreatment is to decompose and destroy the crystalline regions in the lignocellulosic structure; therefore, the cellulose can be easily hydrolyzed for being easier to be accessed by enzymes [17].

Some recent technologies for the pretreatment of lignocellulosic biomass are microwave and ultrasound irradiations. The microwave is widely known as an environmentally friendly heat source in which its radiation can create a magnetic field that oscillates, making polar molecules like water spin as they try to keep up with the changing charges. The friction between molecules results in rapid heating [18]. As a result, lignocellulose pretreatment using microwave is more effective than conventional heating [19].

On the other hand, ultrasound is a wave of acoustic energy with a frequency above the hearing range (>20 kHz) [20]. Ultrasound waves produce acoustic cavitation, spontaneously forming microbubbles. Once they collapse, shockwaves/local hotspots with high temperatures and pressure are created, lying to the disintegration of the crystalline structure [21]. Wang et al. [22] reported that ether linkages in lignin were broken on ultrasound treatment.

Generally, lignin inhibits enzymes from reaching polysaccharides by forming a protective shield around cellulose and hemicellulose. Adding non-ionic surfactants enhances the solubility of lignin, increasing the delignification. Adding Tween 80 as a surfactant to the lignocellulosic pretreatment has given better results compared to Tween 20 and PEG 4000 [23]. Jin et al. [24] reported that, despite its low cost, Tween was a strong surfactant to enhance biomass saccharification by separating polymer walls or increasing cellulase enzyme activity. According to Chen et al. [25], surfactants change the structure of the substrate enabling them to improve the accessibility of enzymes, avoid unproductive bonding between enzymes and lignin, prevent enzyme denaturation during hydrolysis, and increase the positive interaction between the substrate and the enzyme.

So far, no studies are reported concerning with the pretreatment of lignocellulosic biomass by the assistance of microwave followed by microwave- or ultrasound-assisted hydrolysis in the presence of surfactants. In this research, water hyacinth biomass was hydrolyzed using commercial cellulase enzymes with and without the addition of surfactants. The impact of microwave and ultrasonic irradiation on the hydrolysis result was investigated as well.

2. Materials and Methods

2.1. Materials

Water hyacinth biomass was obtained out of a swamp area in Tuntang, Indonesia. The *Aspergillus niger*-based cellulase enzyme with the average activity of ~0.8 U/mg was obtained from Sigma-Aldrich, Germany (Cat. 22178). Dinitrosalicylic acid (DNS) (98%), sodium hydroxide (NaOH) (>99%), sodium sulfite (Na_2S) (>99%), and sodium-potassium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) (>99%) were purchased from Merck, Germany. Tween 20 (CAS-No: 9005-64-5) (>99%), Tween 80 (CAS-No: 9005-65-6) (>99%), and Span 80 (CAS-No: 1338-43-8) (>99%) were obtained from Merck, Germany.

2.2. Pretreatment of water hyacinth biomass

The delignification process was carried out by two pretreatment methods: alkali pretreatment with conventional heating and under microwave irradiation. The samples of water hyacinth biomass were suspended in a 3% NaOH aqueous solution and heated in an autoclave for 15 min at 120°C [26]. The residues were filtered, washed with distilled water until being neutral, dried at 60°C for two days, and weighed. The result of this treatment was denoted as alkali-treated water hyacinth. The delignified water hyacinth fibers were analyzed for their composition, functional groups, crystallinity, and surface morphology.

The alkali pretreatment under microwave irradiation was carried out by dispersing water hyacinth biomass in a 3% NaOH aqueous solution in a beaker glass. The slurry was then put into a microwave oven with a maximum power of 450 W and conducted for 12 min [27]. The products were filtered and prepared as in the alkali pretreatment. The result of this treatment was denoted as microwave-assisted alkali-treated water hyacinth. Moreover, the raw water hyacinth was denoted as the untreated water hyacinth.

2.3. Hydrolysis of water hyacinth biomass

The cellulose from water hyacinth was enzymatically hydrolyzed by following the methods of Jongmeesuk et al. [26], Menegol et al. [28], and Rokhati et al. [29] with modification.

2.3.1. Hydrolysis with shaker incubator

1 gram of water hyacinth that was microwave-assisted alkali-treated was dispersed in a beaker of 50 mL of acetic acid/sodium acetate buffer (pH 4.7) with the concentration of 0.1 M. A certain amount (0 – 12% w/w) of surfactant Tween 80 and cellulase enzyme as much as 1% (w/w biomass) were added to the mixture. Furthermore, it was incubated at 50°C and continuously stirred (100 – 250 rpm) for various times (4 – 24 h). Having completed the hydrolysis, the enzyme was inactivated by putting the beaker into a boiling water bath for 10 min; the mixture was then filtered. Then, the amount of the reducing sugars was estimated by analyzing the filtrate.

To investigate the impact of surfactant types, the addition of Tween 20 and Span 80 was carried out in the hydrolysis process. The procedure was similar with the one previously explained, except that the hydrolysis was conducted for 24 h with a constant stirring (100 rpm).

2.3.2. Microwave-assisted hydrolysis

The reaction mixture was prepared with a similar procedure as in the hydrolysis with a shaker incubator, except that the amount of Tween 80 was 0 and 6%. The mixture was then put into the microwave oven with adjustable power (90 – 270 watts) for 10 min. The resulted mixture was then treated as in the hydrolysis with a shaker incubator.

2.3.3. Ultrasound-assisted hydrolysis

The reaction mixture was prepared with a similar procedure as in the hydrolysis with a shaker incubator, except that the amount of Tween 80 varied (0 – 9%). The beaker was placed onto the ultrasonic generating device at various times (3 – 15 min). The resulting mixture was treated as in the hydrolysis with a shaker incubator.

2.4. Analysis

Following the Chesson method, the delignified water hyacinth fibers were analyzed for cellulose, hemicellulose, and lignin content [30]. To estimate the amount of the reducing sugars of the product that was produced from the enzymatic hydrolysis, DNS method was applied [31]. The DNS reagent was prepared by dissolving 1 g of 3,5-dinitrosalicylic acid (DNS), 0.05 g of sodium sulfite, and 1 g of sodium hydroxide in distilled water to 100 mL and then stored in a dark bottle. Rochelle's salt solution was prepared by dissolving 40 g of sodium potassium tartrate in distilled water to a volume of 100 mL and stored in a closed bottle at room temperature.

1mL of the sample was placed into a test tube and 2 mL of distilled water and 1 mL of DNS were added into the tube and mixed. The test tube was plugged and covered using aluminum foil to keep it out of light. It was then heated in a boiling water bath for 8 min until the solution turned red-brown. The solution was cooled to warm. Next, 1 mL of Rochel's salt solution was added, cooled to room temperature and diluted by adding 20 mL of distilled water. A spectrophotometer was used to measure the absorbance of the solution at λ = 575 nm. A standard curve obtained using a standard glucose solution was used to estimate the total reducing sugars in product. The reducing sugar content was calculated as mg of equivalent glucose units per ml of hydroxylate and expressed in mg/ml.

The Fourier Transform Infrared (FTIR) spectra analysis was conducted to determine the functional groups presented in the raw and treated water hyacinth. The FTIR spectra were recorded using a spectrophotometer IR Prestige-21 Shimadzu in the 500 – 4000 cm^{-1} wavenumber range. X-ray diffraction (XRD) patterns were obtained by Shimadzu Lab XRD-7000 diffractometer with a source of Cu Kα radiation operated at 30 kV and 30 mA. The diffracted intensity was measured at 2θ of 5° to 90°. The surface morphology of raw and treated water hyacinth was obtained by a scanning electron microscope (JEOL JSM-6510LA SEM, Japan) with an acceleration voltage of 10 kV. The samples were coated with platinum before image taking with 5,000' magnificent.

3. Results and Discussion

3.1. Water hyacinth biomass pretreatment

Pretreatment aims to eliminate hemicellulose and lignin from the polymer matrix to make the cellulose easily accessed during enzymatic hydrolysis [15]. Zhu et al. [16] stated that the weight loss and the chemical composition changes of water hyacinth after pretreatment could be used to indicate the effectiveness of the pretreatment process. Figure 2 depicts the remaining weight of the water hyacinth after the treatment. The remaining weight of water hyacinth after alkali and microwave irradiation-assisted alkali treatment was 52.75% and 43.38%, respectively. It showed that microwave irradiation could improve water hyacinth alkali pretreatment, as indicated by a high removal or low recovery.

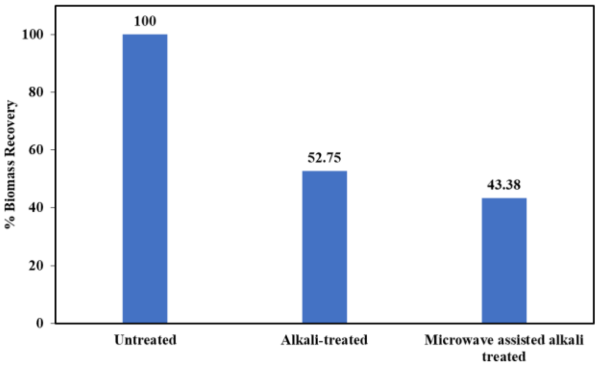

Fig. 2. Effect of pretreatment on the remaining weight of water hyacinth

Besides the removal efficiency, the chemical composition of water hyacinth was also investigated. In general, lignocellulosic biomass mostly consists of 20–35% hemicellulose, 10–25% lignin, 35–50% cellulose, oils, proteins, and trace amounts of other components, including phenolic substituents, acetyl groups, and minerals [32]. In this work, lignin, hemicellulose, and cellulose were estimated using the Chesson procedure in which some residual components such as soluble sugars (pectin and oligosaccharides) and minerals (such as ash) were not calculated [30]. Therefore, the total sum of hemicellulose, cellulose, and lignin was not equal to 100%.

Figure 3 shows the chemical composition of untreated and treated water hyacinth. It showed that untreated water hyacinth had 45% hemicellulose, 22.6% cellulose, and 3.4% (w/w) lignin. After alkali pretreatment, the cellulose increased to 43.97% in contrast to hemicellulose and lignin, decreasing to 43% and 2.03%, respectively. After 12 min of treatment at 450 watts, the microwave-assisted alkali-treated water hyacinth had 39% hemicellulose, 50.44% cellulose, and 1.56% lignin. Water hyacinth's hemicellulose and lignin content decreased after both treatments while cellulose increased. It was caused by hemicellulose and lignin solubilization in the alkali solution, leaving cellulose with a higher content than untreated water hyacinth. Compared to conventional alkali pretreatment, microwave-assisted alkali pretreatment removed more lignin and hemicellulose. Similarly, Zhu et al. [16] reported a similar result. Microwave irradiation may improve the chemical reaction between alkali and biomass. The solubilization of hemicellulose and lignin makes cellulose more accessible to enzymes, thus enhancing enzymatic hydrolysis.

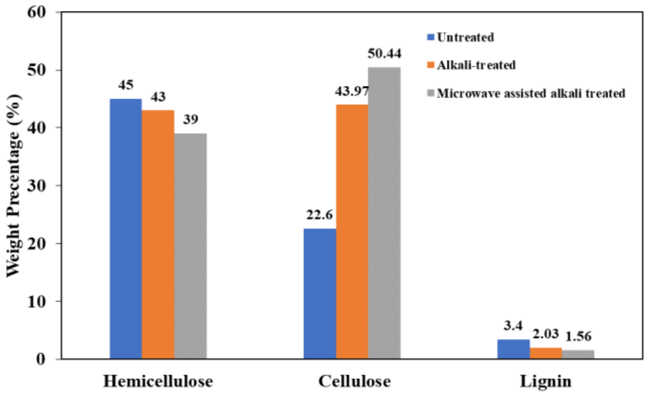

Fig. 3. Chemical composition of water hyacinth fibers

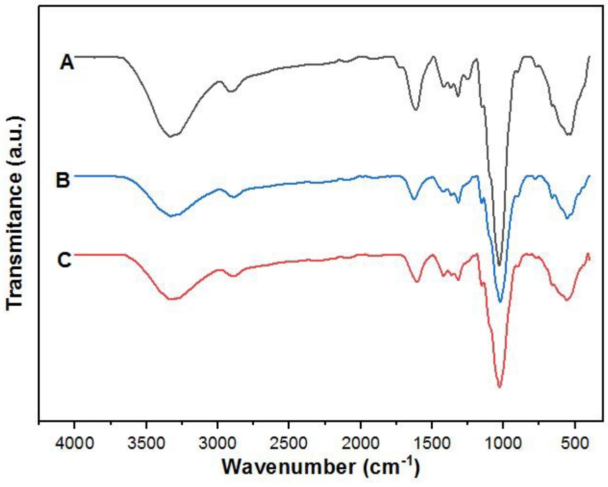

Fig. 4. FT-IR spectra of untreated (A), alkali-treated (B), and microwave-assisted alkali-treated (C) water hyacinth biomass.

Figure 4 shows the FTIR spectra of water hyacinth. There were a number of lowered bands that could be ascribed to lignin, such as the carbonyl group stretching vibration (1728 cm^{-1}) and the aromatic ring vibration (1635, 1600, and 1510 cm^{-1}). These changes in the FTIR spectra were concordant with the removal of lignin identified through chemical analysis (Figure 3). It was found that the stretching of hydroxyl groups (O−H) appeared at 3440 cm^{-1}. These groups were generally found in cellulose, hemicellulose, and lignin. In addition, the absorption band at 2906 cm^{-1} was assigned to vibration of the C−H bond. The changes at a wavenumber of 980 – 1150 cm^{-1} were remarked in the bands ascribed to the core structures of cellulose and hemicellulose. These bands were referred to the overlap of C−O−H elongation that were found in the primary and secondary alcohols, the vibration of glycosidic linkage of C−O−C, and the vibration of C−O−C ring in the hemicellulose structure. The decrease in these bands indicated that the hemicellulose had been removed. It was consistent with the decrease in the hemicellulose content, as shown in Figure 3. This result was similar to an earlier report by Moretti et al. [33].

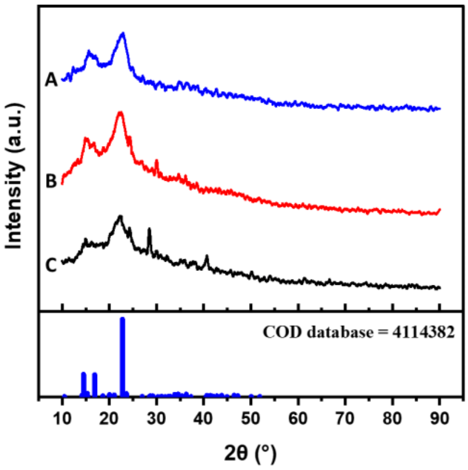

Fig. 5. X-ray diffraction patterns of untreated (A), alkali-treated (B), and microwave-assisted alkali-treated (C) water hyacinth biomass.

The X-ray diffractions of the untreated, alkali-treated, and microwave-assisted alkali-treated water hyacinths are depicted in Figure 5(a, b, and c), respectively. Two peaks were detected at 2θ = 16.5° and 22.54°, corresponding to the crystalline cellulose (COD database = 4114382). As shown in Figure 5(b), the intensity of the highest peak (2θ = 22.54°) was higher than that of in Figure 5(a), indicating the higher crystallinity of cellulose after alkali treatment. It could be attributed to the disposal of non-crystalline components such as lignin and hemicellulose [15,34]. However, Figure 5(c) shows that the intensity of the highest peak was slightly lower than that of in Figure 5(a), indicating that microwave-assisted alkali treatment reduced the crystallinity of cellulose. It could be caused by the non-thermal impact of microwaves, which accelerated the destruction of crystal structures [25].

A distinctive peak at 2θ = ~29° observed in the diffraction pattern of the microwave-assisted alkali-treated sample was associated with a hemicellulose structure. It was similar to Mohtar et al. [35], who reported that the hemicellulose had a peak at 2θ around 29°. It indicated that the pretreatment process could change the crystal structure of hemicellulose and increase its relative crystallinity. Ethaib et al. [36] reported that microwave heating can cause a local breakdown of hydrogen bonds, remove amorphous parts, and cause an increase in the crystallinity index compared to untreated samples.

The surface morphologies of the untreated, alkali-treated, and microwave-assisted alkali-treated water hyacinth were visualized using SEM, as presented in Figure 6. As shown in Figure 6(a), the surface of the untreated water hyacinth was non-porous, smooth, compact, and crystallite. After alkali treatment, the surface became rougher, and several holes were observed, as shown in Figure 6(b). After the microwave-assisted alkali treatment, some erosion of amorphous material from the surface of the biomass could be detected, as depicted in Figure 6(c). It was possibly caused by the decrease in lignin and hemicellulose that were partially removed. This finding is in accordance with Ríos-González et al. [15].

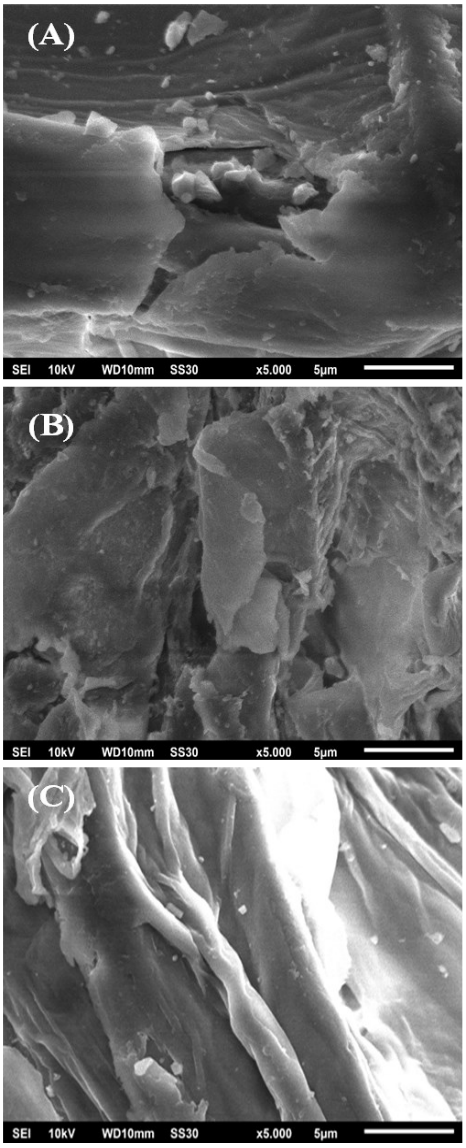

Fig. 6. SEM of untreated (A), alkali-treated (B), and microwave-assisted alkali-treated (C) water hyacinth biomass.

3.2. Hydrolysis of water hyacinth biomass

3.2.1. Hydrolysis with shaker incubator

The impact of reaction time and Tween 80 addition on the resulting reducing sugars is depicted in Figure 7 in which the reducing sugars increased with Tween 80 addition and time. The highest reducing sugar concentration (7.15 g/L) was obtained when Tween 80 was 12% and the reaction time was 24 h. At all reaction times, the reducing sugars increased significantly when the amount of Tween 80 added increased from 0 to 6%. Adding Tween 80 higher than 6% did not significantly affect reducing sugars.

During enzymatic hydrolysis, enzymes attached to a suitable functional group in substrates to form a complex. Subsequent to the reaction, the enzyme desorbed the substrate and adsorbed other functional group of the substrate. The surfactant's presence could affect the enzymes' adsorption and desorption [37]. A low concentration of surfactants created a hydrophilic environment, increasing the desorption rate of enzymes from the functional groups of substrates, thereby increasing the hydrolysis rate. However, the high concentrations of surfactants could weaken enzymes' adsorption onto substrates, thereby inhibiting hydrolysis. Therefore, adding surfactant higher than 6% did not significantly increase the resulted reducing sugars. It is similar to some previous research [12,38,39].

Figure 7 depicts the impact of stirring speed on the resulted reducing sugars. In general, stirring increased the enzyme and substrate mobility enabling them to interact more intensely. In the enzymatic hydrolysis of biomass without surfactants, an increase in the stirring speed lowered the resulted reducing sugars, as shown in Figure 8. Without the presence of a surfactant, the high stirring speed caused a high frictional force that could disrupt the enzyme's three-dimensional structure, leading to enzyme denaturation. According to Okino et al. [40], shear stress resulted from stirring could reduce cellulase enzyme activity.

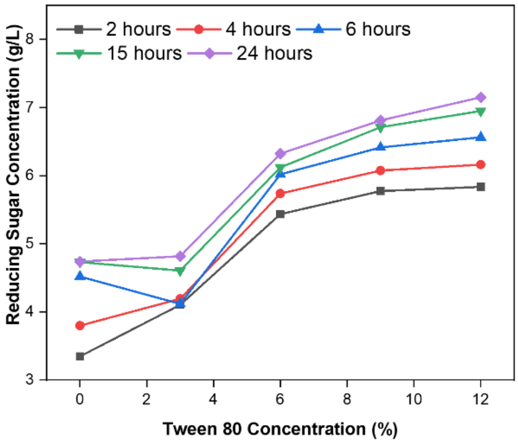

Fig. 7. The impacts of reaction times and Tween 80 concentrations on the obtained reducing sugars in hydrolysis of microwave-assisted alkali-treated water hyacinth biomass with a shaker incubator

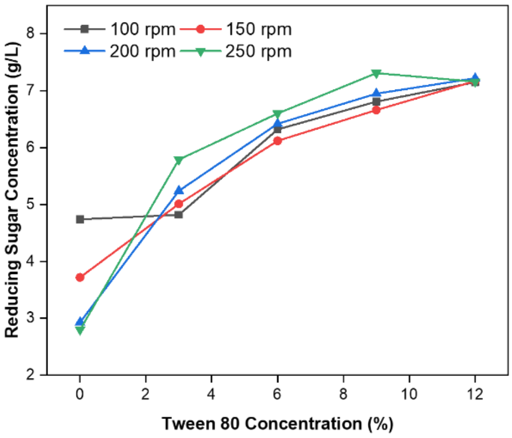

Fig. 8. The impacts of stirring speed and Tween 80 concentrations on the obtained reducing sugars in the hydrolysis of microwave-assisted alkali-treated water hyacinth biomass with a shaker incubator

On the other hand, in hydrolysis, with the addition of surfactants, an increase in the stirring speed can significantly increase the resulted reducing sugars. With the existence of a surfactant, the interaction between the surfactant and the enzyme protein can protect the cellulase enzyme from three-dimensional structure damage. As a result, adding a surfactant to enzymatic hydrolysis at high stirring speeds can increase the reaction rate [12,39].

It is known that the hydrophilic-lipophilic balance (HLB) is a surfactant property that affects the surfactant performance [28,41]. The surfactants with low HLB values surfactants are generally more hydrophobic than that of the high ones, which are generally more hydrophilic [42]. In this work, the surfactants used were Tween 20, Tween 80, and Span 80 with the HLB values of 16.7, 15, and 4.3, respectively [43,44]. Figure 9 shows that the highest reducing sugars was achieved by adding Tween 20, followed by Tween 80 and Span 80. These results showed that increasing the HLB value of surfactants could increase the enzymatic hydrolysis. The increase in the reducing sugars with the addition of surfactant with a high HLB value was caused by the increase in the contact probability. At a high HLB value, the surfactant and substrate could easily interact; therefore, the enzymatic hydrolysis reaction could be increased.

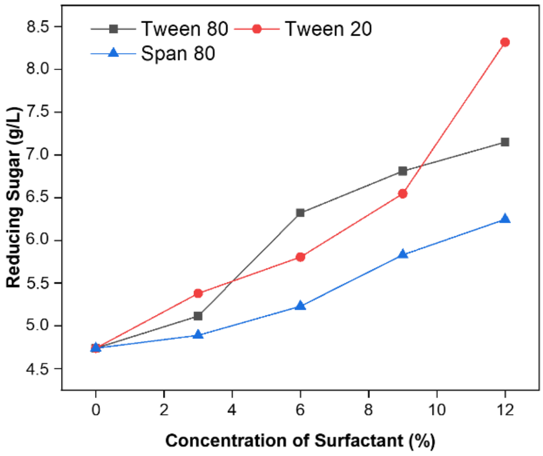

Fig. 9. The impacts of surfactant types and concentrations on the reducing sugar resulting in hydrolysis of microwave-assisted alkali-treated water hyacinth biomass with a shaker incubator

3.2.2. Hydrolysis with the assistance of microwave irradiation

Figure 10 shows the impact of surfactant and microwave power on the obtained reducing sugars in microwave-assisted hydrolysis of microwave-assisted alkali-treated water hyacinth biomass. It was found that the addition of surfactant and higher power of the microwave resulted in a higher amount of the reducing sugars.

The impact of microwave irradiation on hydrolysis can be evaluated by comparing the amount of the reducing sugars resulted in microwave-assisted hydrolysis to the one in conventional hydrolysis. Without the Tween 80 surfactant, the microwave-assisted hydrolysis produced reducing sugars as much as 1.9 – 3.0 g/L in 10 min, dependent upon the microwave power, as depicted in Figure 10. Meanwhile, the reducing sugars of as much as 2.7 – 4.4 g/L, dependent upon the stirring speed, was obtained in 24 h, as observed from Figure 8. It proved that microwave irradiation could enhance the rate of the hydrolysis reaction. The similar trends were observed for the hydrolysis with the presence of Tween 80.

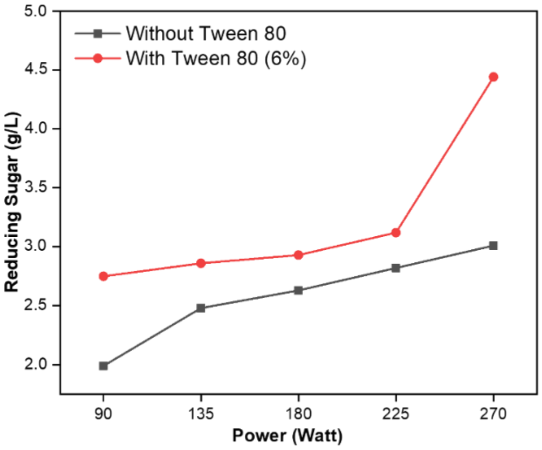

Fig. 10. The impact of Tween 80 addition and microwave power on the obtained reducing sugars in hydrolysis of water hyacinth that was previously microwave-assisted alkali-treated.

Microwave irradiation induces polar molecules to rotate, creating friction between molecules, which can generate heat, causing the rapid heating of the whole material simultaneously [18]. On the other hand, conventional heating is slow, and it originates from the surface to the inside of the material [45]. The higher temperature in microwave-assisted hydrolysis is related to the higher mobility of all molecules in the reacting mixture. Hence, the possibility of collisions among reacting molecules is higher. The rate of reaction, as a consequence, increases.

Shao et al. [46] reported that polar bonds, for example C−O−C glycosidic bonds, could quickly absorb microwave energy and convert it into the energy of motion. Therefore, the glycosidic bonds are stretched by this energy. During the hydrolysis process in the presence of enzyme, these stretched glycosidic bonds are easier to be broken [47].

The quantity of energy transmitted into the reactant is affected by the duration and power of microwave irradiation. Increasing microwave power can increase the photons produced so that the rate of glycoside chain cleavage increases [18].

3.2.3. Hydrolysis with the assistance of ultrasound.

Figure 10 depicts the impact of reaction time and Tween 80 on the hydrolysis of water hyacinth biomass. The resulted reducing sugars of hydrolysis with the presence of Tween 80 was found higher than that of without surfactant addition.

The reducing sugars with a concentration of 3.97 g/L was gained in the ultrasound-assisted hydrolysis with the addition of 6% Tween 80 in 9 min, as shown in Figure 11. A higher concentration of reducing sugar (5.43 g/L) was obtained in conventional hydrolysis, as well as with the addition of 6% Tween 80 in 2 h, as depicted in Figure 6. It indicated the effectiveness of the ultrasound in assisting the hydrolysis of water hyacinth biomass. When an aqueous solution was subjected to ultrasonic irradiation, the cavitation of micro-bubble resulted in instantaneous extremely high pressure and temperature up to 1000 atm and 5000°C, respectively. It then caused the homolytic bond breaking of the water molecule to radical components (hydrogen and hydroxyl). These radicals then collided, forming hydrogen gas and hydrogen peroxide. It is known that hydrogen peroxide is a strong oxidant that can depolymerize polymer (cellulose) by cleaving glycosidic bonds [48]. Hence, ultrasound can enhance the hydrolysis of water hyacinth biomass. It indicates that the ultrasound-assisted hydrolysis performance is determined by the chemical reaction and the physical process (shear force).

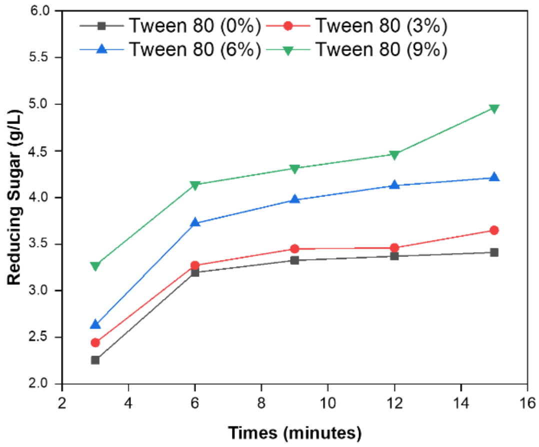

Fig. 11. The impact of Tween 80 concentration and reaction time on the obtained reducing sugars in ultrasound-assisted hydrolysis of water hyacinth that was previously microwave-assisted alkali-treated.

The results presented in Figure 11 also revealed that surfactant positively affected ultrasound-assisted hydrolysis. Karimi et al. [17] reported that the liquid's physical properties, including viscosity and surface tension, strongly affected the cavitation dynamics. Bang and Suslick [49] also reported that a decrease in surface tension could reduce intermolecular forces. The surface tension of water occurs in view of the presence of hydrogen bonds as the result of water molecules interaction in all directions. When surfactants are added to water, the hydrogen bonds on the water's surface reduce, and the tension on the water's surface decreases. As a result, surfactants can reduce water's surface tension, which induces several effects, such as wetting, emulsifying, dispersion, and bubbling [50]. According to Zeng et al. [51], adding surfactant PEG can decrease the surface tension of water and potentially reduces the cellulosic biomass crystallinity; therefore, the cellulose fibers can be easily accessed by cellulase enzymes.

3.2.4. Comparison of microwave- and ultrasound-assisted hydrolysis in the presence of surfactant

Based on the results comprehensively discussed, it is clear that the surfactant-assisted hydrolysis of pretreated water hyacinth is better than non-surfactant-assisted hydrolysis in all hydrolysis systems (conventional, microwave-assisted, and ultrasound-assisted). It is also shown that microwave- and ultrasound-assisted hydrolyses are better than conventional hydrolysis. Therefore, comparing conventional hydrolysis with microwave- and ultrasound-assisted hydrolysis is no longer necessary. It is more reasonable to identify the performance of microwave- and ultrasound-assisted hydrolysis.

With the same amount of Tween 80 (6%) and hydrolysis time (9 min), the ultrasound-assisted hydrolysis produced 3.97 g/L reducing sugar, while the microwave-assisted hydrolysis produced 2.74 – 4.44 g/L dependent on the microwave oven's power. With the microwave irradiation power of 90 – 225 watts, the produced reducing sugar was found lower than in ultrasound-assisted hydrolysis. It indicated that ultrasound-assisted hydrolysis was better than microwave-assisted hydrolysis (Table 1). Ultrasound-assisted hydrolysis performance was caused not only by the chemical reaction but also by the physical process (shear force), as explained above.

On the other hand, microwave-assisted hydrolysis was caused by heat effects. The C−O−C glycosidic bonds can be stretched because they can quickly absorb the microwave energy. Therefore, they can be easily broken during the hydrolysis process in the presence of enzymes. However, at a high microwave irradiation power (270 watts), the microwave-assisted hydrolysis of pretreated water hyacinth was found higher than ultrasound-assisted hydrolysis, indicating a need for high power to increase the reducing sugar production.

Table 1. Comparison of conventional, ultrasound- and microwave-assisted enzymatic hydrolysis of pretreated water hyacinth

Hydrolysis process	Power (Watt)	Hydrolysis time	Reducing sugar concentration (g/L)
Conventional	-	2 h	5.43
Ultrasound-assisted	-	9 min	3.97
Microwave-assisted	90 – 225	10 min	2.74 – 4.44

4. Conclusion

The hydrolysis of water hyacinth utilizing cellulase enzyme with or without surfactant (Tween 80) has been conducted with and without the assistance of microwave and ultrasound. Water hyacinth biomass, meanwhile, was treated using alkali with and without microwave assistance. The results indicated that the alkali treatment with microwave assistance was better than the conventional one in removing hemicellulose and lignin. Tween 80 could significantly increase the resulted reducing sugars in conventional, microwave-assisted, and ultrasound-assisted enzymatic hydrolysis of water hyacinth biomass. In addition, utilizing microwave as well as ultrasound could significantly increase the reducing sugar production in surfactant-assisted hydrolysis. It was found that ultrasound-assisted hydrolysis performance was caused by the chemical reaction and the physical process (shear force); while, microwave-assisted hydrolysis was caused by heat effects. Based on the performance comparison, it can be concluded that ultrasound-assisted hydrolysis was found better than others. The results of this research can be used as the groundwork for further developing the lignocellulosic biomass hydrolysis process.

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