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Digestibility of Betung Bamboo Fiber Following Fungal Pretreatment

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Abstract

This research evaluated the effect of fungal pretreatment of betung bamboo fibers and enzymatic- and microwave-assisted hydrolysis on the reducing sugar yield. The enzymatic hydrolysis of the pretreated biomass was carried out with cellulase and 10 and 20 FPU/g of substrate in a shaking incubator at 50 °C and 150 rpm for 48 h. The sulfuric acid concentration used in the microwave-assisted acid hydrolysis was 1.0, 2.5, and 5%, either with or without the addition of activated carbon. Microwave irradiation (330 Watt) was applied for 5–12.5 min. The yield of reducing sugar was better with the microwave-assisted acid hydrolysis, and the yield tended to increase with an increase in the irradiation time. Based on the dry weight of the initial biomass (bamboo), pretreatment with 5% inoculum loading resulted in a higher reducing sugar yield (17.06%) than with 10% inoculum loading (14.54%). At a 1% acid concentration, the formation of brown compounds decreased, followed by a reduction in the reducing sugar yield. The addition of activated carbon at a 1% acid concentration seemed to be of no benefit with respect to the yield in the microwave-assisted acid hydrolysis. The pretreatment with the 5% inoculum loading for 12.5 min at 1% acid concentration resulted in the highest reducing sugar yield. Under these conditions, the yield was 6.3-fold that of the reducing sugar yield using 20 FPU/g of cellulase. The rate of bamboo hollocellulose hydrolysis reached 22.75% of the maximum theoretical reducing sugar reducing sugar of dry biomass.

Abstrak

Digestibilitas Serat Bambu Betung Setelah *Pretreatment* Jamur. Penelitian ini bertujuan untuk mempelajari pengaruh *pretreatment* jamur pada serat bambu betung terhadap rendemen gula dari hidrolisis enzimatis dan *microwave* dengan asam. Hidrolisis enzimatis pada bambu setelah *pretreatment* menggunakan selulase 10 dan 20 FPU/g substrat dalam inkubator *shaker* pada suhu 50 °C selama 48 jam pada kecepatan 150 rpm. Konsentrasi asam sulfat yang digunakan dalam hidrolisis asam dengan *microwave* yaitu 1,0, 2,5 dan 5% baik dengan atau tanpa penambahan karbon aktif pada daya *microwave* 330 watt selama 5-12,5 menit. Hasil penelitian menunjukkan bahwa hidrolisis asam dengan *microwave* menghasilkan rendemen gula yang lebih baik yang cenderung meningkat dengan bertambahnya waktu iradiasi. Berdasarkan berat kering dari biomassa awal bambu, *pretreatment* dengan konsentrasi inokulum 5% menunjukkan rendemen gula pereduksi yang lebih tinggi (17,06%) daripada konsentrasi inokulum 10% (14,54%). Penambahan karbon aktif cenderung menurunkan pembentukan senyawa coklat. Namun, pada konsentrasi asam 1% penurunan pembentukan senyawa coklat juga diikuti dengan penurunan rendemen gula pereduksi. Tampaknya tidak terdapat pengaruh menguntungkan pada rendemen gula dengan adanya penambahan karbon aktif dari hidrolisis *microwave* dengan asam. *Pretreatment* dengan konsentrasi inokulum 5% selama 12,5 menit pada konsentrasi asam 1% menghasilkan rendemen gula pereduksi yang tertinggi. Rendemen ini meningkat 6,3 kali daripada rendemen gula pereduksi dari hidrolisis enzimatis menggunakan enzim 20 FPU/g selulase dan laju hidrolisis holoselulosa bambu mencapai 22,75% dari rendemen gula pereduksi teoritis dari biomassa awal.

Keywords: activated carbon, betung bamboo, microwave and enzymatic hydrolysis, reducing sugar yield, white rot fungi

1. Introduction

Bamboo is a versatile material, which can be used as a food source and in building materials. Components of bamboo can also be utilized in the production of chemicals, textiles, pulp and paper, and bioethanol. It is a fast growing species, which readily establishes itself once planted [1-2], and it has a higher biomass and a higher productivity than other woody plants [3]. Bamboos have a wide distribution throughout Asia [4]. There are 160 species found in Indonesia, with betung bamboo (*Dendrocalamus asper*) a particularly important species [5].

Although bamboo is thought to have potential has a biofuel, the complex association that exists between hemicelluloses and lignin inhibits the hydrolysis of its cellulose. Therefore, achieving a satisfying degree of hydrolysis requires pretreatment of the lignocellulosic material. Biological pretreatment of lignocellulosic material using white rot fungi (WRF) improved its enzymatic hydrolysis [6,7]. *Trametes versicolor*, a white rot fungus, rapidly grew on bamboo substrate and produced pulp with a lower lignin content than pulp produced from the WRF *Pleurotus ostreatus* and *Phanerochaete crysosporium* [8-9]. The previous works claimed that fungal pretreatment decreased the lignin content of materials and that lignin loss in bamboo following inoculum pretreatment (5%) was higher than 10% [10]. Another study found that the aforementioned pretreatment improved the hydrolysis rate [11].

There have been a number of studies of the production of bioethanol from bamboo. De Menezes et al. [12] and Ram and Seenaya [13] described the development of pretreatment processes used to increase enzymatic hydrolysis rates in bioethanol production from bamboo. Shimokawa et al. [4] obtained ethanol yields of 169 and 139 g kg^{-1} from immature bamboo shoots of *Phyllostachys bambusoides* and *P. pubescens*, respectively, using 12 FPU g^{-1} of cellulase enzyme, with simultaneous saccarification and fermentation. Lignin biodegradation of bamboo by *Cariolus versicolor*, a white rot fungus, effectively enhanced the reducing sugar yield and the hydrolysis rate [7]. Microwave pretreatment of bamboo also increased the reducing sugar and the ethanol concentration by about 2.3% and 8%, respectively [14].

Although enzymatic hydrolysis tends to result in a lower yield, and it is time consuming, it works in mild conditions (a pH of 4.8 and a temperature of 45–50 ° C) and does not cause corrosion [15]. Acid hydrolysis in bioethanol production retains a commercial application. However, this method causes corrosion problems and requires pH neutralization before fermentation.

Microwave-based hydrolysis offers a promising path to effective conversion of lignocellulosic material. It is considered an environmentally benign method, energy efficient, less time consuming [14], and capable of increasing the sugar yield. Microwave irradiation results in a high rate of vibration of polar molecules, causing molecular friction [16]. The direct interaction between the substrate and the electromagnetic microwave field selectively heats the polar ends of the molecules. The addition of organic acids, sulfuric acid, and inorganic acids can enhance the effect of microwave irradiation, depending on the target product [17] and the activated carbon. In microwave irradiation, activated carbon operates as a strong absorbent of the microwave energy. Molecular nonuniformity due to microwave irradiation creates “hot spots” on the surface of activated carbon particles [18]. In a previous study, the addition of activated carbon in microwave hydrolysis of starch pulp increased the glucose yield at a lower heating temperature [19].

The present research determined the reducing sugar yield following enzymatic hydrolysis and microwave-assisted acid hydrolysis of *T. versicolor* pretreated betung bamboo for 30 days. The effect of the addition of activated carbon on microwave irradiation was also studied.

2. Methods

Fresh and barkless 2 year-old betung bamboo culm (*D. asper*) procured from the bamboo garden of the Research Center for Biomaterials, Indonesian Institute of Sciences (LIPI), Cibinong Indonesia was chipped with a drum chipper, dried, and then ground with a hammer mill and a disk mill to produce bamboo powder with a 40–60 mesh size. *T. versicolor* culture was obtained from the Research Center for Biology, LIPI, Cibinong, Indonesia. Cellulase enzyme used in the enzymatic hydrolysis is Meicellase from *Trichoderma viride* (Meiji Seika Co., Ltd., 200 FPU/g, and β-glucosidase activity 264 IU/g).

The bamboo powder (15 g) was watered using a ratio of 1:4 and then stirred until completely mixed. The wet bamboo powder was then put in a jar, steamed for 30 min at ± 100 °C, and finally autoclaved for 20 min at 121 °C and 1 atm.

Microorganism cultivars were prepared by inoculating *T. versicolor* on malt extract agar (MEA) slants (10.65 g of MEA were diluted in 300 mL of destillated water) for 7–14 days. At the end of the incubation period, 5 mL of Japan Industrial Standard (JIS) broth medium (3 g of KH_2PO_4, 2 g of $MgSO_4.7H_20$, 25 g of glucose, 5 g of peptone, and 5 g of malt extract in 1 L of destillated water) were injected into the slants and scratched with a loop to release the resulting mycelium from the agar. Up to 5 mL of the previously prepared fungi suspension was then poured into 95 mL of the JIS broth medium in

a 300 mL elemeter flask and incubated stationery at 27 °C for 7–10 days. After the incubation, 10 g of corn steep liquor were poured into the 100 mL inoculum. The mixture was then homogenized in a high-speed warring blender for 40 sec.

In the pretreatment stage, the culture of *T. versicolor* (5 and 10% w/v of dry bamboo) was inoculated into the medium containing bamboo fibers and incubated at 27 °C for up to 30 days [10]. The pretreated samples were then washed with distilled water to remove the fungus. The residues of these samples were used as substrates in the enzymatic saccarification with cellulase. The enzyme loading was 10 and 20 FPU/g of dried substrate. The enzyme hydrolysis was performed at 50 °C for 48 h in a shaking incubator at 150 rpm, following the Laboratory Analytical Procedure for Enzymatic Saccharification of Lignocellulosic Biomass of the National Renewable Energy Laboratory [20].

The fungal-treated samples were also subjected to microwave-assisted acid hydrolysis. The hydrolysis was carried out in a SHARP P-360J (S) microwave oven, with a frequency of 2450 MHz and the power level was set at 330 Watt. In the microwave-assisted hydrolysis, 0.1 g oven dry weight (1% w/w of total weight) of the fungal pretreated samples was transferred to a Teflon tube (vessel). Then, a sulfuric acid solution (1.0, 2.5, or 5%) was added to reach a final slurry weight of 10 g. The slurry was exposed to microwave irradiation for 5–12.5 min at a given power. Microwave hydrolysis with active carbon (0.5 g of solid sample) was also performed, following the same procedures and hydrolysis conditions described above. The study used granular reactivated carbon produced at 800 °C for 120 min. The properties of the carbon were analyzed based on SNI 06-4253-1996. After the microwave process, the samples were immediately cooled in ice water. The cooled samples were filtered, and the resulting hydrolyzates were used to determine the reducing sugar yield, following the Nelson–Somogyi method [21]. The method of Yu et al. [22] was used to calculate the hydrolysis rate, which is the ratio of the reducing sugar yield to the holocellulose content. This method considers the weight loss during the pretreatment prior to their enzymatic and microwave hydrolysis as a reducing factor. The hololocellulose content was determined following the procedures of TAPPI T9 m-54. The brown compounds of the hydrolyzates were measured with a UV VIS Hitachi U-2001 spectrophotometer at 490 nm [23], and the pH value was measured, in triplicate, with a Eutech pH meter.

The reducing sugar yield was calculated based on the dry weight of the hydrolyzed substrate (fungal pretreated bamboo) and the dry weight of the initial bamboo biomass (nonpretreated substrate) (Eq.1 and 2). All the experiments were carried out in triplicate, and the data are expressed as mean values. The theoretical reducing sugar yield (Eq. 3) was calculated only for the highest reducing sugar yield.

Reducing sugar yield (% hydrolyzed substrate)

$$= \frac{Total\ reducing\ sugar\ (g)}{Dry\ hydrolysis\ substrate\ (g)} \times 100 \qquad (1)$$

Reducing sugar yield (% initial biomass)

$$= \frac{Total\ reducing\ sugar\ (g)}{Dry\ biomass\ (g)} \times 100$$

(2)

Theoretical reducing sugar yield (%)

$$= \frac{Reducing\ sugar\ yield\ (g)}{Carbohydate\ of\ initial\ bamboo\ (g) \times 1.11} \times 100 \qquad (3)$$

1.11=conversion factor of holocellulose to reducing sugar.

3. Results and Discussion

The-assisted microwave hydrolysis resulted in a higher reducing sugar yield than the enzymatic-assisted hydrolysis, indicating that short microwaves with internal heating of the substrate by electromagnetic waves can effectively hydrolyze cellulose into reducing sugar. The cleavage of lignin polymers in the fungal pretreated samples and disruption of the fiber, as confirmed by scanning electron microscopy in Figure 1, helped to increase the accessibility of the substrate in the hydrolysis process.

Based on our parallel study, pretreatment of bamboo with *T. versicolor* for 30 days increased the selectivity of delignification, thereby improving the yield of reducing sugar due to the removal of lignin and subsequent structural changes [10]. The latter increased the susceptibility of the substrate to enzyme and microwave heating. However, in the present study, the yield of reducing sugar following enzymatic hydrolysis of the bamboo substrate was lower than that reported by Hermiati et al. [7] who used oil palm fronds as a substrate and an enzyme mixture of Novozyme cellulase and β glucosidase. The enzyme quality and the chemical composition, anatomical structure, and crystallinity of the substrate all likely influence the resulting sugar yield. In another study, enzymatic hydrolysis of oil palm empty fruit bunches using a coculture of *T. versicolor*, and *P. crysosporium* with 10% inoculum loading for 4 weeks resulted in a higher reducing sugar yield (16.3%) [24] than that found in the present study. In the current study, increasing the enzyme loading slightly increased the reducing sugar yield (Figure 2). However, even with two-fold enzyme loading, only a small quantity of

holocellulose was converted into reducing sugar, as indicated by the hydrolysis ratio (Figure 2). Previous studies reported that fungal pretreatment reduced the lignin content of lignocellulosic material [7,11]. However, the results of the present study indicate that enzymatic hydrolysis of fungal pretreated bamboo fiber does not result in a high yield of reducing sugar. The highest yield of reducing sugar following the enzymatic hydrolysis using 20 FPU/g was only 2.69% or 3.37% of the theoretical reducing sugar yield of the initial biomass. Compared to the control, the reducing sugar yield increased 1.58 fold with enzymatic hydrolysis.

Regardless of the application of activated carbon (Figure 3A and B), the intensity of microwave irradiation improved the reducing sugar yield at both the 5% and 10% inoculum loading pretreatments. The reducing sugar yield increased 9.7-fold compared to the control following microwave-assisted hydrolysis. The yield of the pretreated fungal bamboo was higher than that of the microwave-pretreated bamboo [14]. The present study indicated that the reducing sugar yields of betung bamboo fibers pretreated with the 5% inoculum loading were higher than those pretreated with the 10% inoculum loading. Although the cellulose crystallinity was higher at the 5% inoculum loading than that at the 10% loading (Table 1), more amorphous (lignin and hemicellulose) components of the lignocellulose matrix were removed at the 5% loading. The removal of the

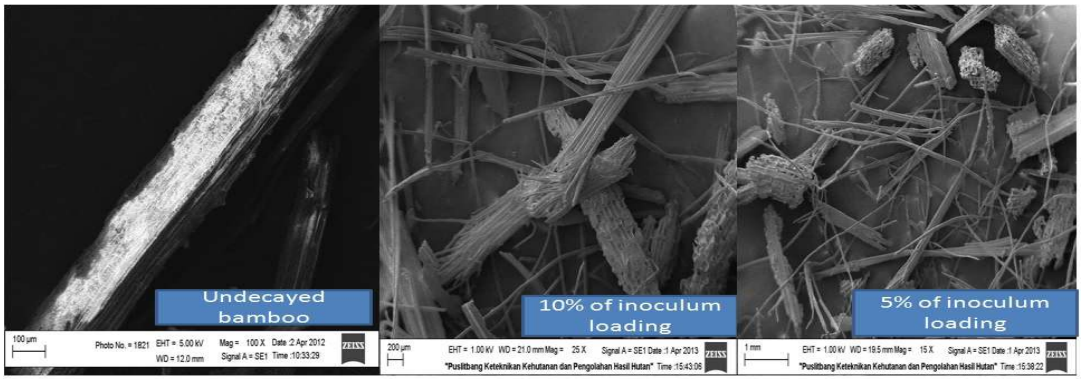

Figure 1. Scanning Electron Microscopy Image of the Fungal Pretreated Bamboo for 30 Days [10]

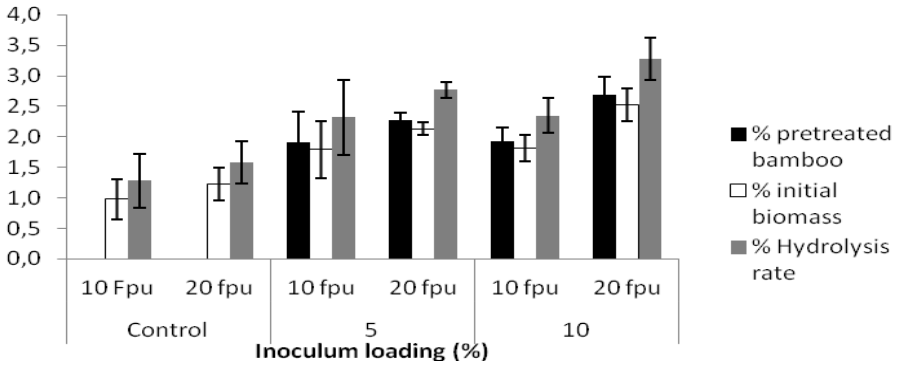

Figure 2. Reducing Sugar Yield and Hydrolysis Rate of Fungal Pretreated Bamboo Following Enzymatic Hydrolysis

Table 1. Fold-Change in the Crystallinity of the Cellulose and Loss of Chemical Components After the Fungal Pretreatment of the Bamboo

Pretreatment	Cellulose crystallinity (%) [10]	Fold-change compared to control (%)	Component loss (%)		
			Lignin [10]	Hemicellulose [10]	Total
Control	30.43				
5% of inoculum loading	38.39	26.16	24.27±2.3	10.92±6.16	35.19
10% of inoculum loading	30.83	1.32	9.78±4.3	16.83±1.3	26.61

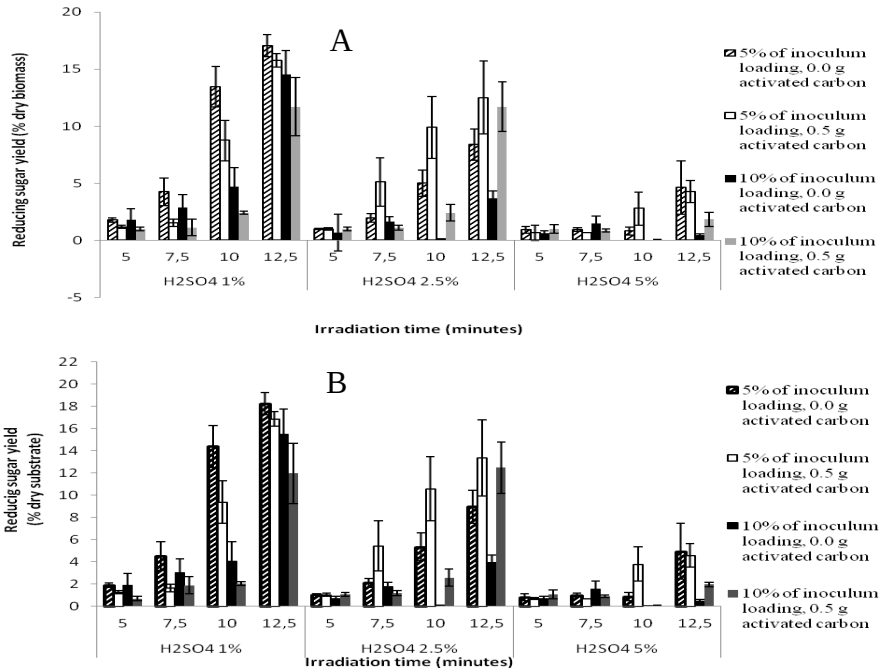

Figure 3. Reducing Sugar Yield Following the Hydrolysis of the Bamboo Pretreated with *T. versicolor* based on the Initial Bamboo (A) and Pretreated Bamboo (B)

amorphous components likely contributed to the increase in the crystallinity of the cellulose. Moreover, to determine the crystallinity of the cellulose, the wet pretreated samples had to be dried. The hornification effect produced by drying the pretreated substrate may have led to the formation of permanent hydrogen bonds, thereby increasing the crystallinity of the cellulose.

Without the presence of activated carbon, increasing the acid concentration in the microwave-assisted acid hydrolysis sharply decreased the reducing sugar yield. More secondary degradation products might form during hydrolysis under severe acid hydrolysis conditions. Lignin removal due to acid treatment of cellulose has been reported to increase the crystallinity of cellulose, thus decreasing the penetration of hydrolyzing agents and the production of reducing sugar [25]. Hermiati [26] reported similar results using starch pulp and microwave irradiation for 12–15 min. Hermiati attributed the findings to the decomposition of glucose to compounds, such as HMF and acid, that have a lower molecular weight.

The addition of activated carbon in the microwave-assisted acid hydrolysis only enhanced the reducing sugar yield at a 2.5% acid concentration, suggesting that the acid concentration was more important than the addition of activated carbon in the process. Hermiati et al. [27] also reported that activated carbon could improve the glucose yield in water medium but not in acid medium. A higher acid concentration prevents the formation of brown compounds in acid medium due to a fast Maillard reaction, which inhibits the formation of glicosilamida [28]. The decrease in the reducing sugar yield in the 1% acid concentration mixture with the activated carbon might be related to the adsorption of malto-oligomers from the surface of the activated carbon, as these that cannot be readily hydrolyzed [27].

The reducing sugar yield improved after microwave hydrolysis using the 2.5% acid concentration for 12.5 min at both the 5 and 10% inocula loadings. Pretreatment with the 5% inoculum loading for 12.5 min using the 1% acid concentration appeared to produce the highest reducing sugar yield (17.06% dry biomass or 18.24% pretreated bamboo), and this yield increased 6.3-fold compared to the reducing sugar yield from enzymatic hydrolysis using 20 FPU/g cellulase enzymes. In this condition, the amount of holocellulose, which can be converted to glucose, was 21.86% (Figure 4). Theoretically, conversion-reducing sugar of bamboo with 100% of hydrolysis rate can produce 71.45 g of reducing sugar/100 g of dry biomass. Therefore, this pretreatment reached 22.75% of the theoretical maximum reducing sugar yield of dry bamboo.

The addition of activated carbon (0.5 g/g) tended to lighten the color of the hydrolyzates, represented by a decrease in the formation of brown compounds. This trend was relatively consistent at the 1% acid concentration. Increasing the irradiation time tended to increase the formation of brown compounds (Figure 5). Although more brown compounds formed at the 1% acid concentration than at the other concentrations, the

reducing sugar yield was better than at the other concentrations. Activated carbon functions as an absorbent material. Therefore, extending the irradiation time increased the degradation of the resulting reducing sugar to brown compounds. The increase in the formation of brown compounds may also be due to the secondary degradation of hemicellulose during acid hydrolysis.

Increasing the acid concentration also reduced the pH value (Figure 6). The formation of organic acids, such as formic acid and acetic acid produced by the secondary degradation of carbohydrates, might be responsible for the decrease in the pH [19,29-30]. Acetic acid is also derived from acetyl groups in hemicellulose [30].

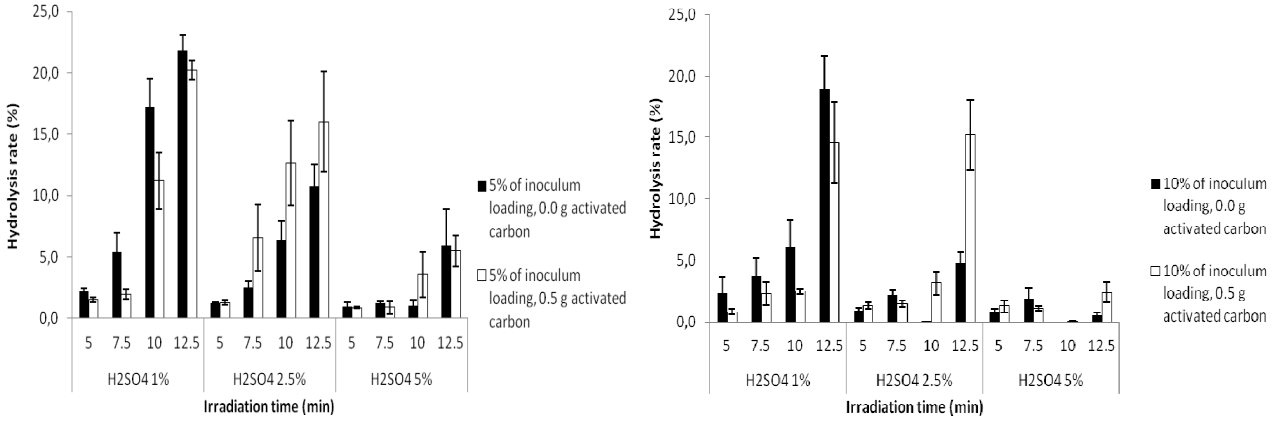

Figure 4. Hydrolysis Rate of Microwave-assisted Acid Hydrolysis

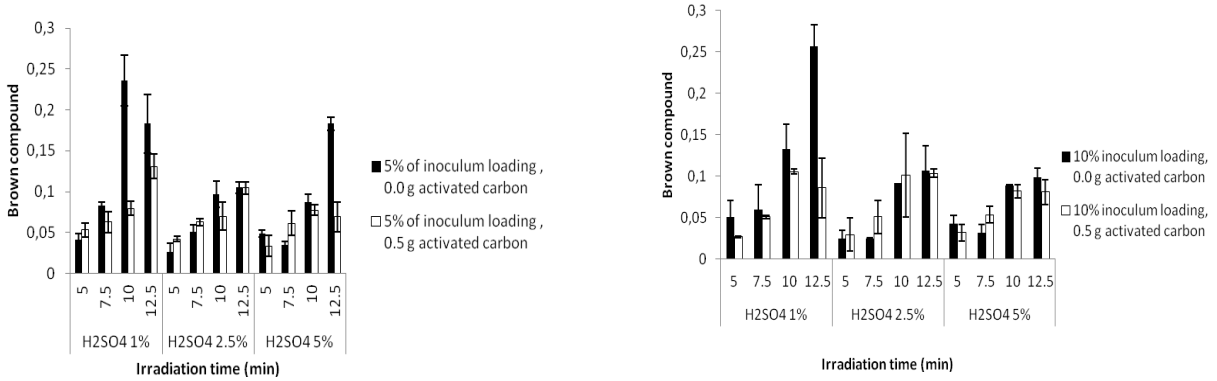

Figure 5. Formation of Brown Compounds During Microwave Hydrolysis of the Pretreated Bamboo

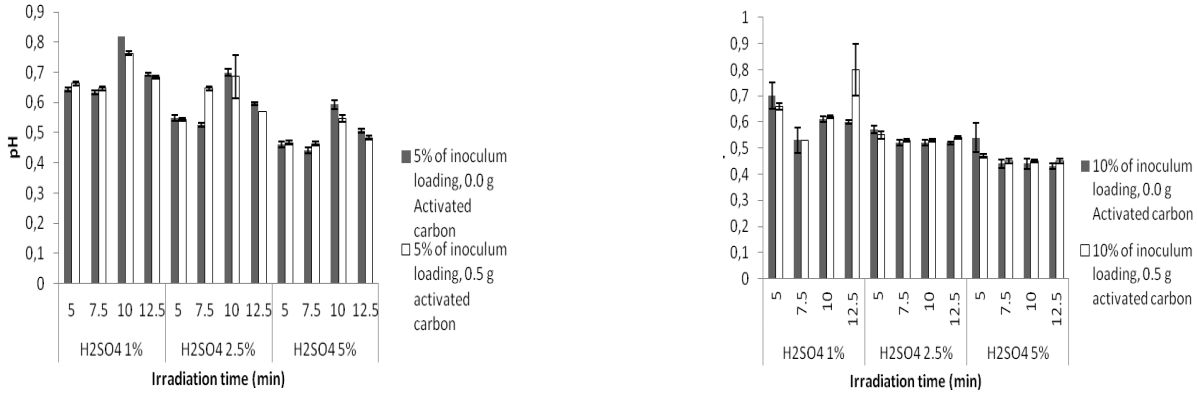

Figure 6. The pH of the Pretreated Bamboo During Microwave Hydrolysis

4. Conclusions

Microwave-assisted acid hydrolysis produced a higher reducing sugar yield than enzymatic hydrolysis. The reducing sugar yield tended to increase in microwave hydrolysis in accordance with an increase in the irradiation time, in both the 5% inoculum and 10% inoculum loading pretreatments. The reducing sugar yield with the 5% inoculum loading pretreatment was higher than with the 10% inoculum loading. Increasing the acid concentration tended to decrease the pH of the hydrolyzate. Although, the addition of the activated carbon in the microwave acid hydrolysis tended to decrease the formation of brown compounds, it also decreased the reducing sugar yield. Thus, activated carbon was not required in the microwave-assisted acid hydrolysis. Pretreatment with the 5% inoculum loading for 12.5 min using a 1% acid concentration gave the highest reducing sugar yield (17.06% of initial bamboo or 18.24% of pretreated bamboo), and this yield increased 6.3-fold compared to the yield with enzymatic hydrolysis using 20 FPU/g of cellulase enzymes. The 5% inoculum loading pretreatment can convert as much as 22.75% of the theoretical maximum of reducing sugar of nonpretreated bamboo.

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