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Effect of Microwave Pretreatment on Some Properties of Bamboo (*Gigantochloa apus*) for Bioethanol Production

Andre Fahriz Perdana Harahap [a,*], Yuli Amalia Husnil [b], Muhammad Yusuf Arya Ramadhana [a], Muhammad Sahlan [a], Heri Hermansyah [a], Bambang Prasetya [c], Misri Gozan [a]

[a] *Bioprocess Engineering Program, Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, Jakarta, Indonesia*
[b] *Chemical Engineering Department, Institut Teknologi Indonesia, Tangerang Selatan 15320, Indonesia*
[c] *National Standardization Agency of Indonesia (BSN), Gedung Badan Pengkajian dan Penerapan Teknologi (BPPT), Jakarta, Indonesia*
*Corresponding author: *andrefahriz25@gmail.com*

***Abstract*— Pretreatment of lignocellulosic biomass plays an essential role in bioethanol production as an alternative biofuel. This process reduces biomass recalcitrance in order to improve cellulose digestibility for saccharification and further fermentation reactions. In this study, microwave (MW) pretreatment was done on bamboo (*Gigantochloa apus*) to investigate the resulting physicochemical properties and bioethanol produced. Bamboo was irradiated in the microwave at different power (100-600 Watt) and irradiation time (5-20 minutes) followed by Water Soluble content (WSC) and lignin analysis. Simultaneous saccharification and fermentation (SSF) using cellulase enzyme was also done in five different treatment combinations (C1-C5) to investigate the effect of produced reducing sugar and bioethanol. The result shows that increasing MW power and irradiation time could decrease WSC gradually. The lowest WSC of 0.3% was obtained at 600-Watt MW power and 20 minutes irradiation time. Lignin content decreased from 18.9% to 16.0% concerning increasing irradiation time from 5 to 20 minutes under 300-Watt MW power. SEM images show that partial disruption and micro-scale pores existed in pretreated samples. The highest amount of ethanol was obtained at 24 hours fermentation for pretreated bamboo at 300-Watt MW power for 15 minutes followed by cellulase enzyme addition. The overall results showed that microwave pretreatment is a prospective method for future bioethanol production from bamboo due to effective WSC reduction and lignin degradation in a relatively short period of time.**

***Keywords*— Bamboo (*Gigantochloa apus*); bioethanol; lignin; microwave pretreatment; reducing sugar; water soluble content.**

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I. INTRODUCTION

The increasing demands on global energy and environmental problems have stimulated efforts for biofuels' production from renewable resources that will be used to supplement gasoline to reduce dependence on fossil fuels and obtain environmental benefits [1]. Ethanol can be blended with gasoline or used as neat alcohol in dedicated engines, taking advantage of the higher octane number and higher heat of vaporization [2]. The main processes in second-generation bioethanol, which uses lignocellulosic biomass as the raw material, include hydrolysis (saccharification) and fermentation. Saccharification is first done to hydrolyze cellulose into glucose through cellulase enzyme, followed by fermentation, where glucose is converted into ethanol through yeast [3]. Simultaneous Saccharification and Fermentation (SSF) is a method to produce bioethanol from lignocellulosic biomass where both processes occur in a single reaction at one time [4]. Bamboo is one of the most abundant natural resources in many countries, particularly those located in Asia. This region accounts for over 65% of the global availability of bamboo forest where India (11.4 million ha), China (5.4 million ha), and Indonesia (2 million ha) are the top 3 bamboo producing countries, followed by America [5]. Bamboo starts to become an alternative bioenergy crop and potentially promising raw material for bioethanol production because of its abundant availability due to rapid growth, perennial nature, high tolerance to extreme climatic conditions, and high cellulose content [6]. Wet chemical analysis shows that bamboo mainly contains cellulose (40.7 %) followed by hemicellulose (26.5%), lignin (27.1%), and ash (1.2%) [7]. This high cellulose content of bamboo can be converted by cellulase enzyme to fermentable sugar, the reactant for bioethanol production in the fermentation process [8]–[10].

Pretreatment of lignocellulosic biomass is necessary to facilitate hydrolysis of its cellulose and hemicellulose fractions, by removing the lignin content, before other processes in common biochemical production [11]. In bioethanol production from lignocellulosic biomass, pretreatment is needed to enhance the accessibility of cellulase enzyme to cellulose and minimize the number of enzyme inhibitors formed during the pretreatment process [12]. Microwave (MW) pretreatment technology is recently used to convert lignocellulosic biomass to sugars and ethanol due to its fast treatment time, selective processing, instantaneous control, and acceleration of the reaction rate [13]. MW-assisted alkali pretreatment of lignocellulosic biomass had been done in previous research using sodium hydroxide as a solvent which could effectively remove 73.75% lignin in oil palm empty fruit bunch [14]. Application of MW-assisted alkali pretreatment of bamboo by using potassium hydroxide as solvent significantly removed bamboo's recalcitrance and allowed enzymatic hydrolysis improved by 8.7 folds and 20.5 folds for glucose and xylose, respectively, compared with untreated bamboo [15].

Like other lignocellulosic biomass, bamboo also has water soluble contents (WSC), which are substances present in the cell such as phenolics, tannins, gums, and coloring matter [16], [17]. The removal of this water-soluble fraction before the pretreatment process had been proven to increase the enzymatic digestibility of pretreated biomass. Application of hot water extraction to remove water-soluble extractives of steam-pretreated spruce could increase its enzymatic digestibility, corresponded to 30 % glucose yield improvement without any significant difference in its holocellulose content as reported by Franko *et al*. [18]. Besides, a study by Zhao and Hu (2019) hypothesized that water soluble content from bamboo, most likely proteins larger than 1 kDa, can negatively affect the enzymatic hydrolysis of cellulose by deactivating the cellulose enzyme and making cellulose regions inaccessible for enzyme active site [16].

A previous study showed that a second-generation bioethanol production plant from rice straw biomass via thermochemical conversion is economically feasible. Thus, another preliminary study on potential bamboo biomass for bioethanol production must be done [19], [20]. This study mainly investigated the effect of MW pretreatment of bamboo (*Gigantochloa apus*) at various pretreatment conditions on its physicochemical properties related to SSF for bioethanol production. The change in reducing sugar and ethanol produced during SSF of the bamboo sample was also examined. The results are supposed to give biorefinery study and industries recommendations in utilizing bamboo, which is an abundant raw material, especially in Asia region countries, as feedstock for biochemical production.

II. Material and Methods

A. Material Collection and Preparation

Bamboo (*Gigantochloa apus*) samples were obtained from the lakeside area behind the Faculty of Engineering Universitas Indonesia, located in Depok, West Java, Indonesia. Young (8-month) and old (16-month) bamboo were both used as raw materials in this study. Bamboo samples were then transferred to the laboratory of the Chemical Engineering Department, Universitas Indonesia. For the MW pretreatment study, bamboo was chopped and cut into chip-sized 2 x 2 x 2 cm then dried in an oven at 150 ºC until a constant weight was reached. On the other hand, for SSF study, dried bamboo was further shredded into fiber size, as shown in Figure 1. Cellulase enzyme (Sigma, USA) for SSF was always kept in the refrigerator under 10 ºC before use. Genetically engineered yeast *Saccharomyces cerevisiae* AM12 for bioethanol production was purchased from Research Center for Biology, Indonesian Institute of Sciences (LIPI). All other analytical grades chemical reagents such as H_2SO_4, $MgSO_4.7H_2O$, $(NH_4)_2SO_4$, HCl, NaOH, Rochelle salt ($NaKC_4H_4O_6.4H_2O$), $Na_3PO_4.12H_2O$, $CuSO_4.5H_2O$, KIO_3, $K_2C_2O_4.H_2O$, KI, $Na_2SO_3.5H_2O$, Na_2CO_3, starch, glucose, alcohol, and yeast extract were purchased from Sigma (USA).

Fig. 1 Bamboo samples used as raw material: dried bamboo fiber (left), wet bamboo chip (middle), and dried bamboo chip (right).

B. Microwave Pretreatment

In this study, some experiments were done to analyze MW pretreatment's effect on some physicochemical properties of bamboo. The first experiment was done to analyze the final WSC of both old and young bamboo after being pretreated with MW in which 10 g of old and young dried bamboo was first irradiated in a domestic microwave type Electrolux EMS3087X at 300 Watt for 10 minutes. Each sample was then soaked in 25 ml of 5% H_2SO_4 for 30 minutes. The second experiment was done to analyze the final WSC of bamboo after being pretreated with MW under different MW power for some time in which 10 g of dried old bamboo was irradiated in the microwave under different MW power (100, 300, 450, and 600 Watt) and irradiation time (5, 10, 15, and 20 minutes). The third experiment was done to analyze both MW and alkali pretreatment's synergism effect using NaOH as solvent. In this experiment, alkali pretreatment was done by soaking 10 g dried old bamboo in 20 ml NaOH at different concentrations (1%, 3%, and 5%) for 30 minutes, and MW pretreatment was done by irradiating the sample in the microwave at 300Watt for 10 minutes. For each NaOH concentration, the bamboo sample was soaked first then continued with MW different irradiation time (5, 10, 15, and 20 minutes) where 10 g of dried old bamboo was irradiated in the microwave at 300 Watt.

C. Simultaneous Saccharification and Fermentation

Five treatment combinations (C1-C5) in terms of MW pretreatment condition and cellulase enzyme addition for SSF of bamboo were done in this study, as shown in Table 1. For each combination, 10 g of dried bamboo fiber was used as raw material for SSF. The pretreated bamboo fiber was put in 150 ml demineralized water in an Erlenmeyer flask. Nutrients that consisted of 0.05 g/L $MgSO_4.7H_2O$, 1.0 g/L $(NH_4)_2SO_4$, and 2.0 g/L yeast extract were added into the substrate and mixed well. The flask was then sealed with cotton and autoclaved in a pressure chamber at 120 ºC for an hour and cooled to room temperature. Into the flask was finally added 0.008 g cellulose enzyme and 3.3 g/L yeast *Saccharomyces cerevisiae* AM12. The addition of enzymes and yeast were performed in a sterile box using aseptic procedures. Sample from the fermentation broth was taken every 24 h for 96 h and analyzed for reducing sugar and ethanol content.

TABLE I
TREATMENT COMBINATIONS FOR SSF STUDY OF BAMBOO

Treatment	C1	C2	C3	C4	C5
Microwave power 300 Watt for 10 minutes	✓	×	✓	×	×
Microwave power 300 Watt for 15 minutes	×	×	×	✓	✓
Addition of cellulase enzyme	×	✓	✓	×	✓

D. Physiochemical Analysis

WSC of the bamboo sample was analyzed gravimetrically in which MW pretreated sample from each experiment was transferred in an Erlenmeyer flask and soaked in demineralized water with a solid to liquid ratio equals to 1: 2.5 for 10 minutes while being shaken in an orbital shaker at 30 rpm. After that, the bamboo sample was removed from the flask and dried in an oven at 150 ºC until a constant weight was reached. The final WSC of the bamboo sample was calculated with equation (1) below.

$$\%WSC = \frac{(Weight_{pretreated\ sample} - Weight_{oven\ sample})}{Weight_{pretreated\ sample}} \times 100 \quad (1)$$

The lignin content of the bamboo sample was analyzed according to TAPPI Standard T 222 [21]. One gram of MW pretreated bamboo chip was placed in a beaker glass. Then 15 ml of cold 72% H_2SO_4 was slowly added into the beaker and stirred constantly. The beaker was then placed in a 20 °C water bath, and the shaker was turned on for 2 hours. Afterward, the sample was transferred into a round flask and diluted with 560 ml of distilled water. Allihn condenser was attached to the flask tip, and this set of equipment was then placed in boiling water for 4 hours. When finished, the round flask was taken out from the boiling water and then cooled down at room temperature. The flask's content was then filtered using Whatman filter paper No. 42, and the solid residue obtained was finally dried in an oven at 150 ºC until a constant weight was reached. The lignin content of the bamboo sample was then calculated with equation (2) below.

$$\%\ Lignin = \frac{Weight_{oven\ residue}}{Weight_{pretreated\ sample}} \times 100 \quad (2)$$

The analytical method used to calculate the amount of reducing sugar is the Somogyi method that has been modified [22]. Reagent A was made from 90 g Rochelle salt and 225 g $Na_3PO_4.12H_2O$, which were dissolved in 600 ml demineralized water. Then 30 g $CuSO_4.5H_2O$ and 3.5 g of KIO_3 were dissolved in 100 ml demineralized water, respectively. These solutions were mixed into a 1-liter measuring flask, and the solution is diluted with demineralized water. Reagent B was made from 90 g $K_2C_2O_4.H_2O$ and 40 g KI, dissolved in 1 liter of demineralized water. Na-thiosulfate 0.1 N solution was made from 24.82 g $Na_2SO_3.5H_2O$ and 0.2 g Na_2CO_3, which are dissolved in 1 liter of demineralized water. After all, reagents were ready, 5 ml sample and 10 ml reagent A were mixed into a 100 ml Erlenmeyer flask forming a turquoise transparent solution. The solution is then diluted to 30 ml and heated on a hot plate. The solution is then allowed to boil for 3 minutes and cooled immediately using cooling water. Then 10 ml reagent B and 10 ml 2 M HCl were added to the solution to form a dark brown turbid solution. This solution is then immediately titrated with 0.1 N Na-thiosulfate to form a blue transparent solution.

E. Instrumental Analysis

Bioethanol content from the fermentation broth was analyzed using Gas Chromatography (GC) model 6890 from Agilent Hewlett Packard (USA) equipped with Flame Ionization Detector (FID). Simultaneously, morphology images of pretreated bamboo were analyzed using Scanning Electron Microscope (SEM) type Hitachi SU-3500. All instrumental analysis in this study was done at the Indonesian Institute of Sciences (LIPI) located in Serpong, Tangerang, Indonesia.

III. RESULTS AND DISCUSSION

A. Effect of MW Pretreatment on WSC

In this study, the effect of irradiation time on WSC was analyzed for both young and old bamboo at 300-Watt MW power. From Figure 2 below, it can be seen that WSC of both young and old bamboo decreased gradually when irradiation time was increased from 5 to 20 minutes. The lowest obtained WSC of young and old bamboo in this study was 5.7% and 4.4%, respectively, after being irradiated for 20 minutes. The results also clearly showed that the WSC of young bamboo was higher than the WSC of old bamboo. WSC or also well known as extractive as a nonstructural component of bamboo was also affected by this MW-induced heating in which more severe MW pretreatment tended to remove higher extractives. The decreasing content of extractives in bamboo is favorable for bioethanol production in the SSF process because extractives have potency to inhibit hydrolytic enzymes such as cellulase enzymes [23].

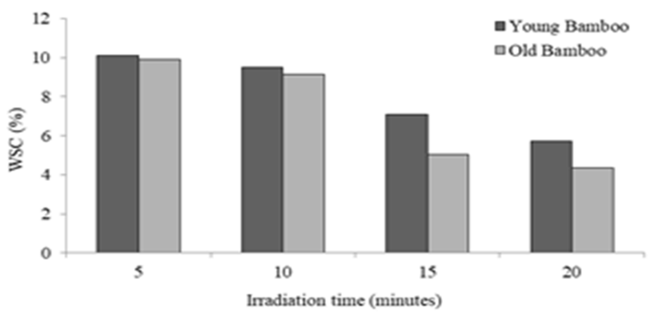

Fig. 2 WSC of young and old bamboo samples after being irradiated at 300-Watt MW power.

The effect of irradiation time and MW power on WSC of old bamboo can be seen in Figure 3 below. The results showed that increasing irradiation time and MW power could decrease the WSC of bamboo samples gradually. The lowest WSC of bamboo sample, as much as 0.3%, was obtained at

600-Watt MW power and 20 minutes irradiation time. From the graph in Figure 3, when the bamboo samples were pretreated for 5 and 10 minutes, increasing MW power from 300 Watt to 450 Watt could significantly decrease the WSC of bamboo samples. The graph also indicates that the MW pretreatment of bamboo at 100-Watt MW power was not effective enough to decrease WSC.

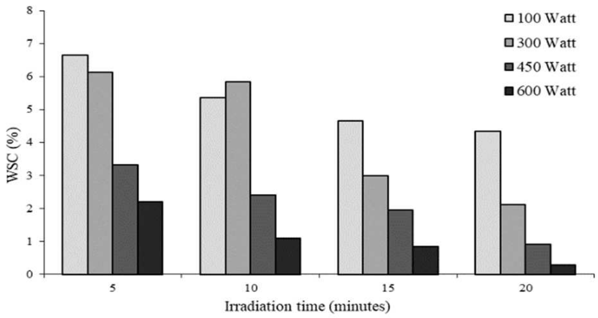

Fig. 3 Effect of irradiation time on WSC of bamboo samples under various MW power.

The synergistic effect of MW alkali pretreatment of old bamboo sample at 300-Watt MW power for 10 minutes was also analyzed in this study. From Figure 4 below, it can be seen that soaking bamboo samples before MW pretreatment was more effective in decreasing WSC than soaking it after MW pretreatment. The graph also indicates that increasing alkali concentration from1 to 5% could gradually decrease the WSC of bamboo samples. The lowest obtained WSC of bamboo samples soaked in 5% alkali before and after MW pretreatment at 300-Watt MW power for 10 minutes in were 5.3 and 8.1%, respectively.

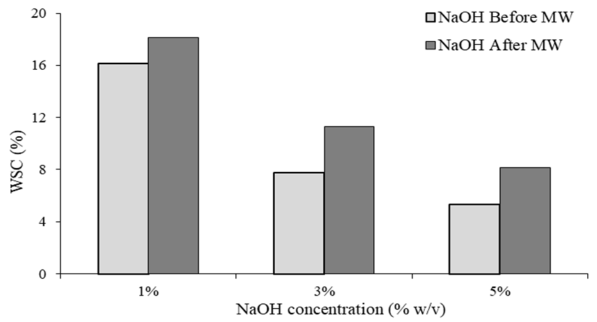

Fig. 4 WSC comparison of bamboo samples soaked in alkali at different concentration before and after being irradiated at 300-Watt MW power for 10 minutes.

B. *Effect of MW Pretreatment on Lignin Content*

From Figure 5 below, the lignin content of unpretreated bamboo was 19.9%. This result is less than the result obtained by Li *et al.* (2012), which was 24.29% due to different bamboo species used in each other's studies [15]. The graph shows that the bamboo sample's lignin content decreased linearly from 18.9% to 16.0%, or 15.3% lignin removal, concerning increasing irradiation time from 5 to 20 minutes under 300-Watt MW power. The graph in Figure 5 shows that the relationship between irradiation time and lignin content is inversely proportional to constant MW power. The lignin removal obtained in this study is higher than that obtained by Fatriasari *et al*. (2016), which was only 10.0% for MW pretreated bamboo (*Dendrocalamus asper*) in distilled water at 550 Watt MW power and 12.5 minutes radiation time [23]. Microwaves are absorbed by water and polar components of biomass, causing rapid heating, which produces some hot spots that can improve the disruption of the lignocellulosic structure of biomass. The microwave radiation also generates a changing magnetic field resulting in the vibration of polar bonds, accelerating the destruction of the crystal structure and lignin degradation [24]. The result implies that MW pretreatment of bamboo can be an alternative pretreatment method for bioethanol production, which effectively degrades lignin in a relatively short period, thus saving more energy.

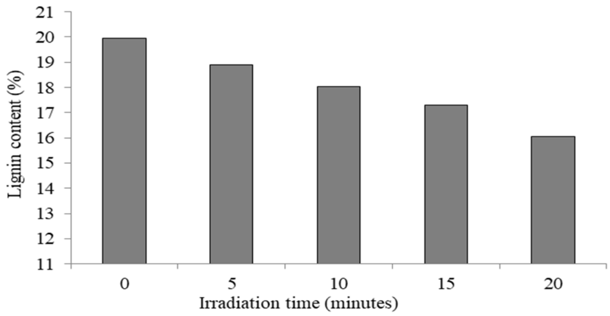

Fig. 5 Lignin content of bamboo samples after being irradiated at 300-Watt MW power.

C. *Effect of MW Pretreatment on Physical Structure*

Scanning Electron Microscope (SEM) images of untreated and treated bamboo samples were shown in Figure 6 below. These SEM images were useful parameters to visually observe morphological characteristics and surface change of untreated and treated bamboo samples. Figure 6 shows that partial disruption at the micro-scale happened for all bamboo samples, even for the untreated ones. The disrupted structure of untreated bamboo was highly likely to occur due to the shredding process for sample preparation. SEM images of MW pretreated bamboo show fiber partial degradation and morphological change where micro-scale pores and cracks were formed on the surface of pretreated bamboo samples. Both the number of micro-scale pores and the disruption intensity increased with the increasing irradiation time.

On the other hand, pretreated bamboo in 5% NaOH showed less disrupted structure without any pores formed. The observed structural damage of pretreated bamboo indicated disruption on carbohydrate-lignin network, partial breakdown of lignin structure, and hemicellulose removal [25]. Intense frequency of the dielectric system during MW pretreatment caused the solvent to diffuse into the molecules of fibers, leading to agitation within molecular structure and then the fibers rub against each other, generating friction by reason of the electric field produced by MW energy [26]. The polar part of water under high MW irradiation generated the heat and encouraged more removal of amorphous bamboo parts (lignin and hemicellulose). At the molecular level, MW irradiation enhances the saponification reaction of intermolecular ester bonds cross-linking and other components, such as lignin and hemicellulose [23]. The hot spot effect caused by MW irradiation facilitated the breakage of C-H and C-O bonds in bamboo with low energy [27]. This condition is very favorable for further cellulose conversion to bioethanol due to higher cellulose accessibility and lower substrate recalcitrance.

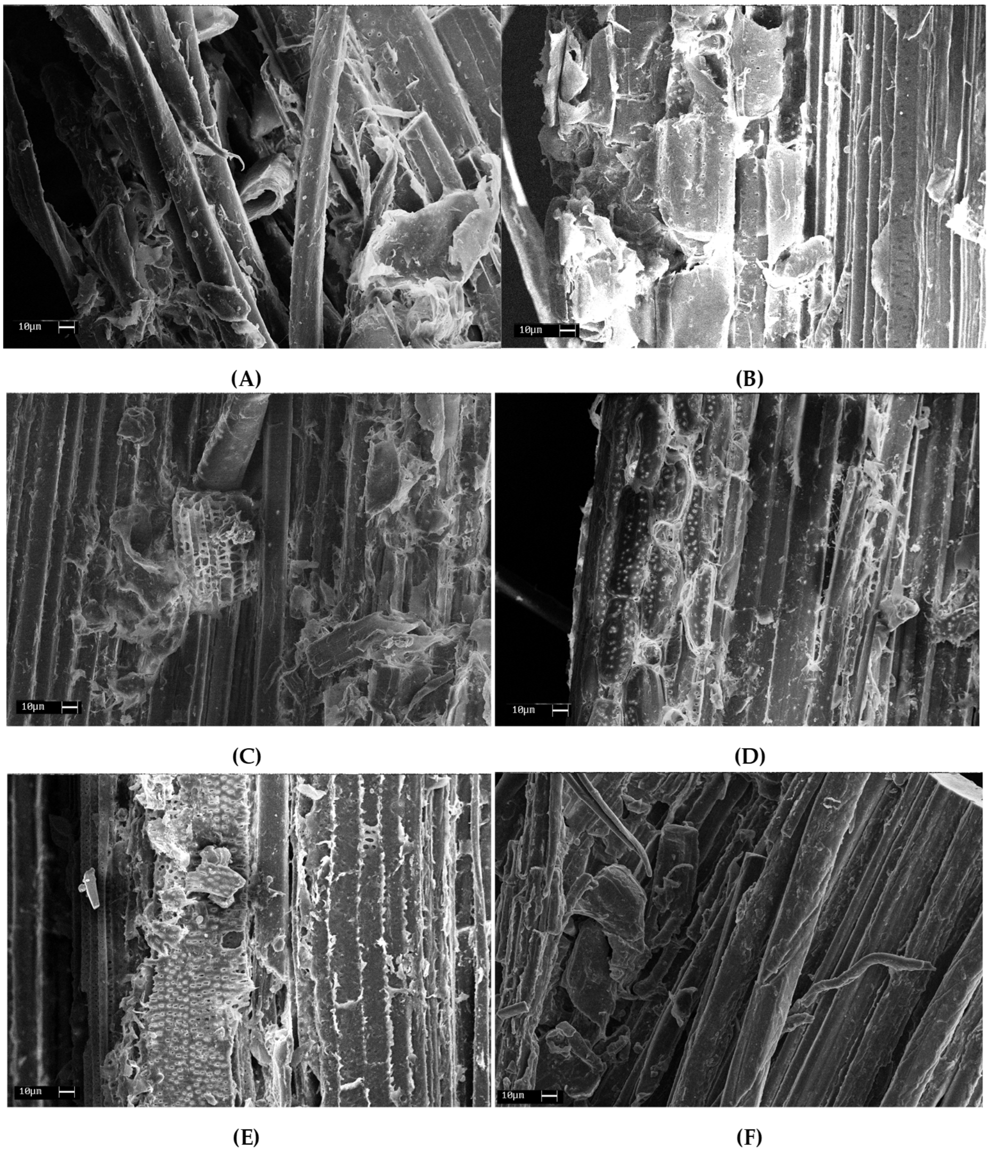

Fig 6. SEM photograph (magnification 500x) of bamboo samples after 300-Watt MW treatment: (A) 0 min; (B) 5 minutes, (C) 10 minutes, (D) 15 minutes, (E) 20 minutes, and (F) 5% NaOH treatment

D. Effect of MW Pretreatment on SSF

Amount of ethanol produced during SSF at different time for all five treatment combinations are shown in Figure 7 below.

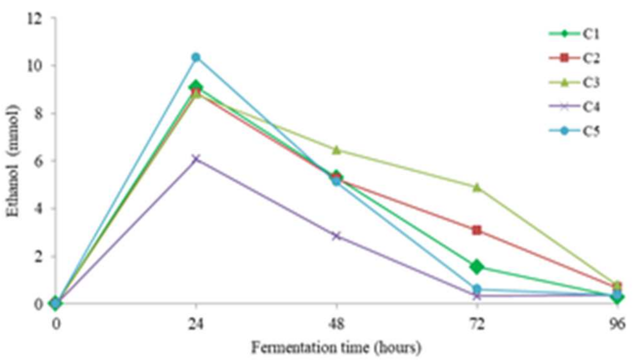

Fig. 7 Amount of ethanol during SSF of bamboo samples under different treatment combinations

From Figure 7, it can be seen that the highest amount of ethanol, as much as 10.3 mmol was obtained at 24 hours fermentation for pretreated bamboo sample under 300-Watt MW power for 15 minutes followed by addition of cellulase enzyme (C5). While the lowest amount of ethanol at 24 hours fermentation as much as 6.1 mmol was obtained for a pretreated bamboo sample at 300-Watt MW power for 15 minutes but without the addition of cellulase enzyme (C4). After 24 hours of fermentation time, ethanol content for all treatment combinations decreased gradually toward zero at 96 hours fermentation time.

The amount of reducing sugar of C5 decreased significantly from 0.910 to 0.235 mmol at 24 h fermentation time and finally approached 0.107 mmol at 96 h fermentation time. This phenomenon improves that both saccharification and fermentation coincide. This process's main objective is to decrease the degree of polymerization of cellulose by hydrolyzing the large polysaccharides to simple sugar yeast to produce bioethanol [28]. The graph in Figure 7 is in line with the previous study by Gul *et al.*, where the highest concentration of bioethanol from Kallar grass by *Kluyveromyces marxianus* was obtained at 32 h fermentation time, and the concentration tends to decrease [29]. This result indicates that bamboo can produce bioethanol optimally after the combination of MW pretreatment and hydrolysis by cellulase enzyme were done.

IV. CONCLUSIONS

Microwave pretreatment is a prospective method for future bioethanol production from bamboo biomass due to effective WSC reduction and lignin degradation in a relatively short period. An increase in irradiation time during microwave pretreatment of bamboo would gradually decrease its water-soluble content and lignin content. The lowest water soluble content of the bamboo sample in this study, as much as 0.3%, was obtained at 600 Watt microwave power and 20 minutes irradiation time. Simultaneously, the bamboo sample's lignin content decreased linearly from 18.9% to 16.0% concerning increasing irradiation time from 5 to 20 minutes under 300-Watt MW power. SEM images of pretreated bamboo under microwave irradiation also show fiber partial degradation and morphological change where micro-scale pores and cracks were formed on the surface of pretreated bamboo samples. Longer microwave irradiation time was found to cause greater fiber disruption due to more effective lignin polymer degradation and hemicellulose removal in the cell wall. Thus, increasing cellulose digestibility by cellulase enzyme for bioethanol production. Results support this from SSF experiment in this study. The combination of pretreatment under 300-Watt MW power for 15 minutes and cellulase enzyme yield yielded the highest amount of ethanol, as much as 10.3 mmol at 24 hours fermentation time.

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