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Development of Bioethanol from Bamboo Shoots Using Hydrogen Peroxide-Acetic Acid (HPAC) and Ultra High-Pressure Extraction (UHPE) Pre-treatment

Izz Qayyum Karim[1], Nasrul Fikry Che Pa[1]*

1Department of Chemical Engineering Technology, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia, 84600 Pagoh, Johor, MALAYSIA

*Corresponding Author Designation

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Abstract: Bamboo is an ideal feedstock for bioethanol production owing to its significantly faster growth rate and widespread and sustainable availability in the tropics. *Gigantochloa albociliata* is a Poaceae bamboo tree and shrub that produces bamboo shoots called *rebung madu*. Due to its natural abundance, high growth rate, perennial nature, and minimal maintenance needs, bamboo has the potential to be an exciting feedstock for advanced bioethanol production. To demonstrate biomass's potential for bioethanol production, pre-treated *Gigantochloa albociliata* bamboo shoots were examined as a feedstock for bioethanol production. The pre-treatment of biomass using Hydrogen Peroxide-Acetic Acid (HPAC) and Ultra High-Pressure Extraction (UHPE) reveals changes in the shape of the surface. The data indicate that the bamboo particles inflated, and the bamboo micropores widened. Thermal stability was determined for both native and treated biomass using a Thermogravimetric Analyzer (TGA), which revealed the existence of cellulose, hemicellulose, and lignin. After obtaining bioethanol from each native and treated sample, the absorbance and transmittance of the biomass samples were determined using ultraviolet-visible spectroscopy (UV-vis) and Fourier-transform infrared spectroscopy (FTIR). Each piece's spectra reveal the existence of ethanol peaks at 1060 to 1001 cm^{-1} and an absorbance of 550 nm. As a result of this study, it is anticipated that bamboo has a high potential for producing bioethanol with an effective pre-treatment process and the addition of raw material alternatives for bioethanol production.

Keywords: *Gigantochloa albociliata*, Bamboo, Bioethanol

1. Introduction

Bioethanol is chemically known as C_2H_5OH ethyl alcohol and is produced by fermentation of fermentable sugars derived from plants using microorganisms. When bioethanol is discharged into the

*Corresponding author: nfikry@uthm.edu.my

environment, it transforms into a clear, colorless liquid that is biodegradable, toxic, and produces tiny pollutants. The present emphasis of bioethanol research is on the generation of lignocellulosic biomass. The most common use of bioethanol research is to fuel automobiles with gasoline.

The technique manufactured bioethanol from lignocellulosic biomass with a higher recovery rate. This may be accomplished in two ways: by pre-treating lignocellulosic biomass to improve its hydrolysis readiness or by fermenting glucose and xylose into bioethanol to raise the conversion yield lignocellulosic biomass to bioethanol. Pre-treatment methods include HPAC and UHPE, which should be cost-effective, efficient in dignifying various lignocellulosic materials, low in cellulose breakdown, and provide negligible inhibitors for subsequent enzymatic saccharification and fermentation.

Numerous earlier research on lignocellulosic biomass has proven that it produces more bioethanol per ton (L/ton) than most commercially available bioethanol feedstocks. The conversion efficiency must be increased to create any amount of ethanol similar to that produced by sugar- and starch-containing material. The mix of components capable of being converted to glucose substantially affects the amount of ethanol paid per ton of feedstock. Due to a large amount of glucose convertible material and availability, these lignocellulosic biomasses are a promising feedstock for bioethanol production.

The objective of this study is to provide the physical changes of the bamboo morphology structure surface before and after being treated with acidic and alkalinity chemical, HPAC and UHPE pre-treatments. Next is to compare the effectiveness of HPAC and UHPE pre-treatment in producing bioethanol from the bamboo shoots. Lastly, this study will provide the proof of ethanol present in the treated and native *Gigantochloa albociliata* bamboo shoots.

2. Literature review

In traditional Chinese medicine, bamboo shoots stimulate uterine contractions, which aids in labour placenta ejection. Shoot poultices are commonly used to clean wounds and treat infections. A decoction of bamboo shoots and honey is used to cure respiratory problems. Bamboo shoots have much potential for health since they are high in proteins, amino acids, carbs, and a variety of essential minerals and vitamins [1]. Malaysia is home to around 70 bamboo species, 50 of which are found in Peninsular Malaysia, 30 in Sabah, and 20 in Sarawak [2]. The available genera are *Bambusa*, *Chusquea*, *Dendrocalamus*, *Dinochloa*, *Gigantochloa*, *Phyllostachys*, *Racemobambos*, *Schizostachyum*, *Thrsostachys* and *Yushania* [3].

The growing interest in renewable energy sources, particularly biofuels, is fueled by increasing fossil fuel costs, increased demand for energy, and environmental concerns. Any fuel derived from plant materials is referred to as a "biofuel." Conventional biofuel methods include well-established processes for manufacturing commercially accessible biofuels [4]. Ethanol is derived from sugar and starch, biodiesel from oil crops and raw vegetable oil, and anaerobic digestion biogas is derived from bacteria. While advanced biofuel technologies enhance previous approaches, some are still in the research and development, pilot, or demonstration phases and are referred to as second or third-generation biofuel technologies.

Pre-treatment is likely the most crucial stage since it has a considerable influence on bioconversion efficiency. Pre-treatment is used to disrupt the structure of refractory cellulosic biomass, enabling enzymes to convert polysaccharides polymers to fermentable sugars. Without using high temperatures or strong acids, the suggested HPAC pre-treatment eliminates lignin. It is compatible with a wide variety of lignocellulosic materials, reduces enzyme loading and subsequent enzymatic hydrolysis time, and decreases the production of fermentation inhibitors. HPAC's novel strategy entails combining H_2O_2 and CH_3COOH to create a reagent capable of extracting lignin from lignocellulosic biomass by partial lignin bond hydrolysis. HPAC is a very efficient and effective method of converting lignocellulosic biomass to fermentable sugars. [5].

The UHPE is a novel method for enhancing mass transport phenomena that operate at super-high pressures ranging from 100 to 500 MPa and temperatures ranging from 20 to 50°C. It has been recognised by the US Food and Drug Administration as an environmentally friendly technology [6]. The efficiency of UHPE is highly dependent on the operating conditions chosen, as these have a significant effect on the extraction mechanisms and yields. The pressure level, the holding time, the ratio of liquid to solid, the temperature, the moisture content, and particle size of plant samples, as well as the type and concentration of solvent, can all affect the performance of UHPE. Understanding these factors' effects and interactions on the UHPE processes [7].

3. Methodology

3.1 Bamboo shoots extraction

Gigantochloa Albociliata was collected from Rimbun Rezeki Garden & Nursery at Parit Jawa, Muar that supplies the bamboo shoots. The bamboo shoots were cut into pieces around 5 cm long. Then the bamboo shoots were air-dried for one night. After one night, the bamboo shoots were dried in an oven for three days at 72 °C. The dried *Gigantochloa Albociliata* that shown in Figure 1 were ground using a dry blender until the models turned into sturdy powder. The samples were divided into three categories which are for natives, HPAC, and UHPE pre-treatments.

Figure 1: The bamboo shoots before and after being dried for 3 days

3.2 HPAC and UHPE pre-treatment of bamboo shoots

HPAC and UHPE pre-treatment will break down the bamboo shoots into cellulose, hemicellulose, and lignin. For UHPE, the treatment starts with preparing the NaOH solution by weighting 3.75- grams of NaOH powder and soluble in 500 ml distilled water to gain 2.00 % of NaOH solution. Then soaked 50 grams of samples into 400 ml of 2.00 % NaOH solution. The soaked model was pressured in an autoclave for 2 hours at 121 °C. The samples were filtered to gain their residue and washed using distilled water until the pH of the samples was nearly neutral at 7.5 pH. The samples were dried oven for three days at 72 °C. The pieces were kept in a plastic jar and stored in the chiller.

HPAC diluted 35.00 % of Hydrogen Peroxide into 1.00 % concentration. Then, weight 0.025 grams and soluble into 250 ml distilled water to gain 1.00 % acetic acid. Then both solutions were mixed to form 1000 ml 1.00 % HPAC solution required for the pre-treatments. After the solution was ready to use, soaked 50 grams of bamboo shoots sample into 500 ml HPAC solution. The pieces were kept in the incubator for 2 hours at 80 °C. The sample residue was washed with distilled water until the pH was

nearly neutral, about 6.7 pH. The samples were dried in an oven for three days at 72 °C. After that, the piece was kept in a dry plastic jar and stored in the chiller.

3.3 Fermentation and bioethanol recovery

Enzymatic saccharification of dilute acid pretreated bamboo was performed in 150 ml stoppered conical flasks by incubating 1.4 grams of dilute acid pretreated biomass in 100 mM citrate buffer (pH 4.8) and 0.1 percent (w/w) surfactant, which was supplemented with 1% (v/v) Penicillin-Streptomycin solution to prevent contamination. Hydrolysis was performed using a commercial cellulase. The samples were incubated for 48 hours at 50 °C, 200 rpm in a shaking water bath. Following enzymatic saccharification, centrifuged samples were utilized to eliminate un-hydrolyzed residue.

Following enzymatic saccharification, the hydrolysate was centrifuged (4 °C, 10,000 g) to remove any remaining unhydrolyzed residue. The hydrolysate was fermented in screw cap vials holding 20 ml hydrolysate. This was seeded using an *S. cerevisiae* seed culture that was 18 hours old and grown at 30 °C for 72 hours. The supernatant was filtered via 0.4 m filters and analyzed using FTIR. The ethanol concentration was calculated by calculating the elution time and comparing it to previously measured ethanol attention [8].

Figure 2: Ethanol recovery using rotary evaporator

A rotary evaporator as shown in Figure 2 was employed to recover the ethanol. After powering on all of the equipment, choose ethanol from the panel's data bank and set the temperature to the boiling point of ethanol. After a few hours of partial immersion, ethanol may be produced.

3.4 Measurement and analysis

Several characterizations of *Gigantochloa albociliata* native and treated samples were measured using ultraviolet-visible spectroscopy (UV-vis), Thermogravimetric analyzer (TGA), Scanning Electron Microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR) to prove the presence of ethanol in the models and the effectiveness of pre-treatments involved.

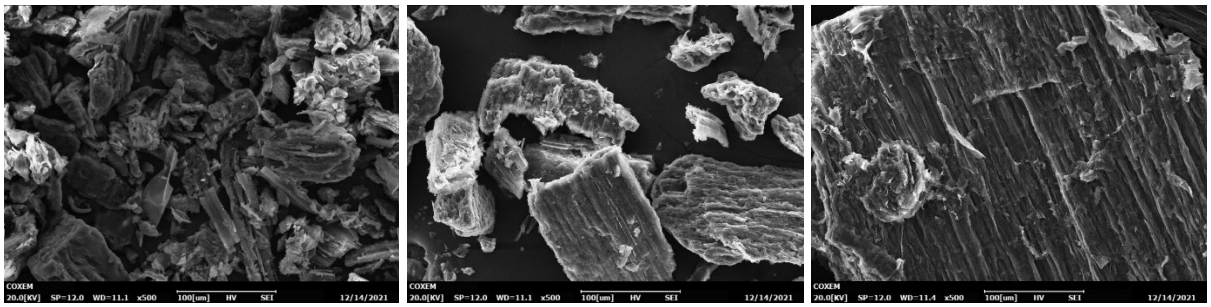
SEM examination was performed using a JOEL JSM-6390 field emission SEM operating at a 5 kV accelerating voltage and 500 magnifications. Meanwhile, a nitrogen gas flow rate of 150 ml/min was used for TGA analysis, along with a continuous heating and cooling rate of 10 °C/min throughout a temperature range of 25 to 600 °C. The wavelength of ethanol is reported to be between 400 and 550 nm in the UV-vis range. The samples were measured for every wavelength until the positive value of

absorbance was shown to indicate the ethanol present in the samples. Each sample was pipetted into the cuvette and using 2 ml of distilled water as a blank. The absorbance measurement was made. The frequency range for FTIR measurements is 4000 to 400 cm^{-1}, with a spectral resolution of 0.5 cm^{-1}.

4. Results and Discussion

4.1 Surface of native and pre-treated samples of *Gigantochloa albociliata*

Biomass pre-treatment is a critical barrier in biomass to bioethanol conversion since it is one of the most expensive and energy-intensive procedures in the whole conversion process. Using SEM, structural changes in the *Gigantochloa albociliata* were determined during pre-treatment. The surface morphology of native and pre-treated bamboo shoots was compared, and it was discovered that the pre-treatments cause the fibres to stretch and the surface area to increase. It is feasible to improve enzyme binding and hydrolysis by increasing the spaces between fibres [9].



(a) (b) (c)

Figure 3: SEM images showing structure modifications resulting from (a) Native (b) HPAC and (c) UHPE sample at 500 magnifications

Structural changes in the biomass during pre-treatment were analyzed using SEM. A comparison of the surface morphology of native and pre-treated *Gigantochloa albociliata* bamboo shoots indicated that UHPE and HPAC method used does give much effect. Based on [10], NaOH effectively disrupted the robust structure of the raw materials relative to H_2SO_4. NaOH that was used in UHPE dissolves bulk lignin and disrupts the initial fibre structure, leading to the disaggregation of micro-fibrils from their neighboring fibre. The structure of the bamboo after pre-treatment was more ordered, delicate, and smooth and appeared like a light emitting structure as shown in Figure 3.

4.2 Thermal stability of native and pre-treated *Gigantochloa albociliata*

TGA was used to determine the thermal stability of native, HPAC, and UHPE-treated samples. TGA may calculate the proportion of volatile components by measuring the weight change while a sample is heated at a consistent pace.

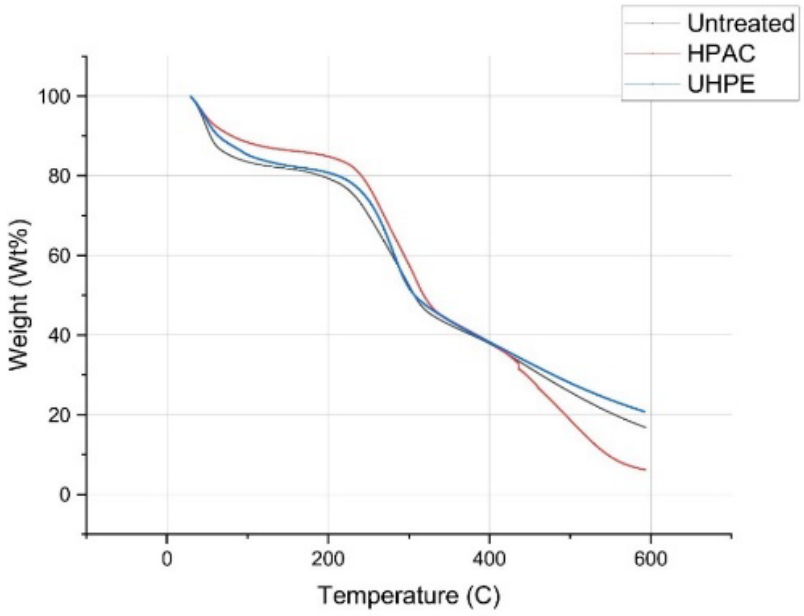


Figure 4: TGA curves of individual bamboo shoots treated by UHPE and HPAC

The TGA curves presented in Figure 4 demonstrate that both native and treated samples lost weight in two different phases between 30 and 150 °C and 200 to 600 °C, respectively. The first weight loss step was attributable to water evaporation, while the second stage of weight loss occurred between 200 and 600 °C due to the degradation of polysaccharides, including cellulose, hemicellulose, and lignin.

4.3 Analysis of samples via UV-vis

After samples were distillate using a rotary evaporator, the bioethanol produced was analyzed using UV-vis. The absorbance gained from the analysis was recorded.

Table 1: Analysis of UV-vis of bioethanol produced from *Gigantochloa albociliata* treated with HPAC and UHPE

Wavelength (nm)	Absorbance		
	Native	HPAC	UHPE
400	-0.064	-0.035	-0.070
450	-0.035	-0.021	-0.033
500	-0.043	-0.029	-0.035
550	0.039	0.11	0.032

The result analysis of UV-vis shows that ethanol is present at 550 nm for all samples. The absorbance gained from the UV-vis is supported with the IR spectrum obtained for each model.

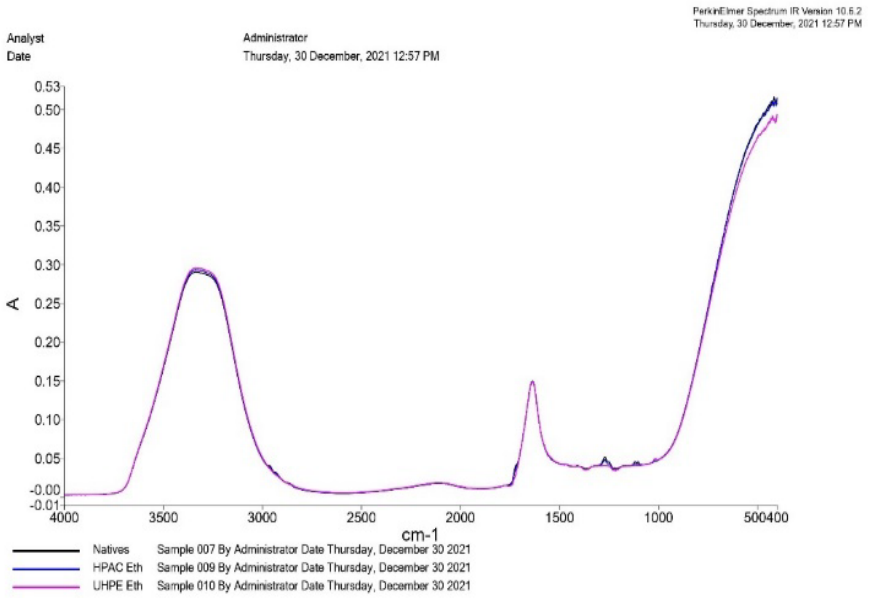


Figure 5: The absorbance of IR spectrum for all samples

Based on Figure 5, the spectrum shows a small absorbance peak at 1060 to 1001 cm^{-1} since the region are classified for ethanol and glucose. Absorbance indicated the amount of light absorbed by the samples' material. The result from UV-vis and FTIR shows the absorbance to prove the presence of ethanol.

4.4 Structural characteristics of samples using FTIR

The structural elements of the polysaccharide sample were recorded on an FTIR. The examples of pre-treated Gigantochloa albociliata were analyzed for FTIR measurement in the frequency range 4000 to 400 cm^{-1}, with a spectral resolution of 0.5 cm^{-1}.

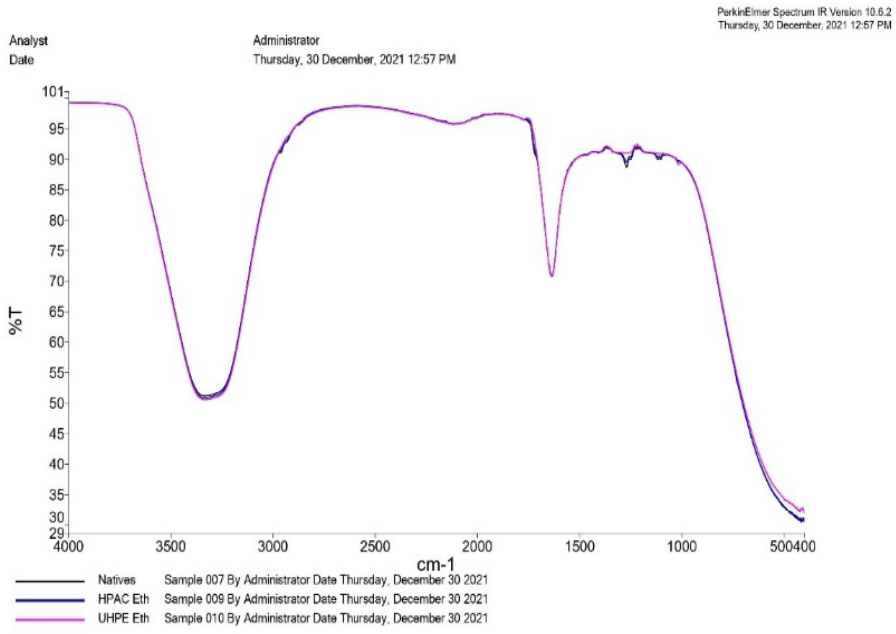


Figure 6: Spectrum of bioethanol from *Gigantochloa albociliata* with HPAC and UHPE pre-treatment

The FTIR analysis of *Gigantochloa albociliata* bamboo shoots pre-treated with HPAC and UHPE revealed many characteristic bioethanol peaks. The adsorption spectrum in Figure 6 illustrates the mountains between the ranges 3400–3200 cm^{-1} (hydroxyl group), 2356–2322 cm^{-1}, 1658–1638 cm^{-1} (alkene group), 1384–1377 cm^{-1}, and 1060–1001 cm^{-1} (alkene group) (ethanol and glucose). Comparing the FTIR findings to those from earlier research [11], it was determined that the peak between 3400 and 3200 cm^{-1} corresponds to the hydroxyl (OH) group in the samples. Current data demonstrate a similar tendency. Thus, ethanol emerges in the model decisively. Additionally, the absorption wave between 1658 and 1638 cm^{-1} indicates the presence of the alkene group, which contains atoms with varying C=C bonds. The peak at 1060 and 1001 cm^{-1} corresponded to the presence of ethanol and glucose in the wave areas between 1200 and 800 cm^{-1} owing to C-O and C-C stretch vibration adsorption bands, respectively. Additionally, the peak between 1100 and 900 cm^{-1} was indicative of the presence of carbohydrates. Thus, FTIR analysis measured the functional group present in bamboo shoot bioethanol.

5. Conclusion

The research study has successfully shown the effect of UHPE and HPAC pre-treatment on *Gigantochloa albociliata* bamboo species by determining the surface morphology of the samples after being treated. This study also identified the characteristics of *Gigantochloa albociliata* samples with or without pre-treatment via TGA. This research has proven the presence of ethanol in *Gigantochloa albociliata* HPAC and UHPE-treated samples by discovering its absorbance and structural characteristics of polysaccharides by using UV-vis and FTIR. For recommendation, further studies should be carried out by including the concentration of pre-treatment medium as a parameter that can be more precise to discover *Gigantochloa albociliata* most significant potential in bioethanol production. The studies also need to include various bamboo species that have great potential in producing ethanol.

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