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High-Pressure Enzymatic Hydrolysis to Reveal Physicochemical and Thermal Properties of Bamboo Fiber Using a Supercritical Water Fermenter

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Bamboo fiber was treated using a high-pressure enzyme hydrolysis process. The process performance was compared with the pulping and bleaching process for bamboo fiber. Several analytical methods, including field emission scanning electron microscopy, Fourier transform infrared spectroscopy, X-ray diffraction, thermogravimetry, and differential scanning calorimetry, were employed to determine the physicochemical and thermal properties of the treated cellulosic bamboo fiber. It was found that the pressurized enzyme hydrolysis treated bamboo fiber had the most uniform morphological structure, along with lowest crystallinity and highest thermal stability. Thus, utilizing high-pressure enzyme hydrolysis is the most effective process for treating fiber to remove non-cellulosic components from the raw material, including lignin, hemicelluloses, and waxy materials.

Keywords: Bamboo fibers; Cellulose; Pressure water; enzyme hydrolysis; Thermal properties; Morphological characterization

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INTRODUCTION

The growing environmental pollution concern, coupled with the increasing scarcity of natural resources, has induced a growing demand for natural fiber reinforced composites (Abdul Khalil *et al*. 2010a,b; Lu *et al*. 2013). Plant fiber reinforced green composites have incited interest among researchers in recent years due to their potential advantages over conventional synthetic reinforced fibers. For example, some reinforced green composite advantages include easy processing, low energy consumption, low cost, light weight, excellent specific strength, low environmental hazard, renewability, and recyclability (Ku *et al*. 2011; Abdul Khalil *et al*. 2013a). Therefore, plant fibers originating from natural resources are being considered a promising candidate for replacing conventional synthetic reinforcing fibers in composites for semi-structural and structural applications (Islam *et al*. 2013).

Cellulose is considered one of the most abundant natural fibers in the world. Numerous studies have been conducted on the utilization of cellulosic plant fiber in various fields. Testing has affirmed that cellulosic plant fiber can act as a reinforcement agent in functional composites, as well as in both thermoplastic and thermosetting polymers (Abdul Khalil *et al*. 2007, 2013b; Melo and Santos 2009; Bhat *et al*. 2010;

Acharya and Samantarai 2012; Sri Aprila *et al*. 2014). Over the years, there have been efforts to find the ideal cellulosic resource among the various plant fibers. One possible solution, bamboo, is recommended as a superior source of cellulose fibers that possess relatively small microfibrillar angles as well as high cellulose content (Abdul Khalil *et al*. 2012a).

Bamboo is an admirable candidate with respect to sustainable natural fiber composite development due to its rapid growth rate and the excellent mechanical properties of bamboo fiber (Wahab *et al*. 2012). There are 50 bamboo species available in Malaysia, of which at least 14 species are known to be commercially utilized (Wong 1989). The *Gigantochloa* genus of bamboo is one of the most popular tropical species because of its thick culms wall, ease of cultivation, and uniformity in the size from node to internode, making the genus a good choice for industrial usage (Mustafa *et al*. 2011).

Cellulosic bamboo fiber has been found to improve certain properties of a given polymer composite matrix. Research on the utilization of cellulosic bamboo fiber in the development of bamboo reinforced composites has grown rapidly in recent years. Studies have reported that the fracture toughness and impact behavior of reinforced composite materials increase with the addition of cellulosic fibers, while the flexural strength increases minimally (Shih 2007; Abdul Khalil *et al*. 2010b; 2012b; Jawaid *et al*. 2014). The fact that the flexural strength does not increase more substantially may be attributed to the poor compatibility between the cellulose and matrix, which is the major limitation of the application of cellulose in reinforced composites materials. Dense hydrogen bonds between the molecules and intra-molecules in the cellulose structure, coupled with its strong polarity, leads to weak matrix accessibility. The poor matrix accessibility increases the interfacial tension between the cellulose fibers, which results in an increase in the porosity of the composites (Reddy *et al*. 2013; Jawaid *et al*. 2014). To improve the mechanical properties of the bamboo fiber composites, researchers have made numerous attempts to overcome poor matrix accessibility, including the pretreatment of the cellulosic fiber by means of physical, chemical, or enzyme hydrolysis (Abdul Khalil *et al*. 2013b; Alwani *et al*. 2014; Dunghani *et al*. 2014). However, the physicochemical and thermal properties of cellulosic fiber are not fully exploited in polymer composites. In recent years, supercritical fluid technology, which utilizes water as a fluid, has been used to modify cellulose prior to its use in composites. Although significant improvements have been made, this process is considered expensive due to high processing costs and energy consumption.

The present study was conducted to determine the feasibility of high pressure enzyme hydrolysis of cellulosic bamboo fiber using a supercritical water fermenter. The fermenter treatment at high pressure could not disrupt the cellulosic crystalline structure, but it could break down the hemicellulose and lignin; and thereby the cellulose can easily access the matrix. Moreover, the physicochemical and thermal properties of the treated cellulosic fiber were analyzed, and the results of the performance were compared with the pulping (mechanical) and alkaline peroxide bleaching (chemical) processes.

EXPERIMENTAL

Materials

Dried bamboo chips (*Gigantochloa scortechinii*), (size 1.5 cm^2) with an overall moisture content of approximately 5%, were obtained from the Forest Research Institute

of Malaysia (FRIM), in Kepong, Selangor, Malaysia. Enzyme "Marugoto A" (Cellulase, from soybeans) was obtained from Supercritical Technology Research Corporation (Nishi-ku, Hiroshima, Japan). The chemicals used in this study were analytical grade and were used as provided by the supplier (Bumificient Sdn Bhd, Malaysia) without further purification.

Methods

Soda-AQ pulping

The pulping of raw bamboo was performed in an "Ibsutek Zat 92" (RB Supply Enterprise, Penang, Malaysia) 20-L stainless steel rotary digester with 25% NaOH and 0.1% anthraquinone (AQ) as cooking liquor. The cooking temperature and time were 170 °C and 3 h, respectively, and the ratio of cooking liquor to bamboo fiber was 7:1. After pulping, the bamboo fibers were washed with distilled water to eliminate chemicals (NaOH and/or AQ), acquired during the cooking process. Finally, the samples were dried in an oven and kept in a desiccator for further processing.

Alkaline-peroxide bleaching

The bleaching of the bamboo pulps was performed accordingly using H_2O_2 and NaOH. The pulps were treated with 3% H_2O_2 (35% v/v), 3% NaOH (0.33 M), and 0.5% $MgSO_4$ (0.039 M) at 80 °C for 2 h. Upon completion of the bleaching process, the samples were rinsed with water. Subsequently, oven-drying was done for 24 h at 60 °C.

High-pressure enzyme hydrolysis

Control of hydrolysis temperature is typical during the enzymatic process (Ku *et al*. 2011). Therefore, high-pressure water hydrolysis using a supercritical water fermenter (Toyokoatsu Co., Ltd.; Hiroshima, Japan) followed the temperature recommended for enzymatic hydrolysis. Prior to treatment, 5 g of dry bamboo fiber and 2.5 g of enzyme were taken into a plain nylon vacuum bag (Et Shoppe Enterprise, Selangor, Malaysia). Subsequently, 500 mL of water was added to the sample, and the vacuum bag was immediately sealed to avoid contamination. Later, the prepared sample was replaced in the fermenter vessel, and the lid of the vessel was closed. The temperature was set at 70 °C, and when the temperature in the vessel reached the desired temperature, water was introduced using a pump at a pressure of 90 MPa. The treatment was run for 6 h, and the treated fibers were filtered and washed with water. Finally, the fibers were dried in an oven and kept in a desiccator for further characterization.

Characterization

The morphology of the samples was analyzed *via* field emission scanning electron microscopy (FE-SEM) (EVO MA10; Carl Zeiss SMT, Germany). The acceleration voltage was set at 15 kV, and the fibers were coated with gold prior to analysis. To determine diameter of the treated fibers, 100 measurements were taken using image analysis software (Image Pro Plus ver.7.01; Media Cybernetics, Inc., USA), and the results were expressed as average diameters from the 100 measurements. Changes in functional groups that may be caused by various treatments of fibers were determined by Fourier transform infrared (FT-IR) spectroscopy (Nicolet iS 10 FT-IR Spectrometer; Thermo Scientific, USA). At first, the samples were ground and dried in an oven for 24 h at 60 °C. Seven milligrams of the fibers was mixed with KBr to obtain approximately 100 mg of sample.

The role of KBr was to hold the fiber flour during the test. Transparent pellets were prepared from the blend and analyzed from 400 to 4000 cm^{-1}.

Structural analysis of the sample was performed using an X-ray diffractometer (D8 Advanced; Bruker, Germany) at 2θ range (5° to 50°). The percentage crystallinity of the cellulose, C_{ir}, was estimated according to the method proposed by Segal *et al*. (1959),

$$C_{Ir}(\%) = \frac{I_{200} - I_{AM}}{I_{200}} \times 100 \quad (1)$$

where I_{200} signifies amorphous and crystalline fractions and I_{AM} is the amorphous region.

The degradation temperature and thermal stability of the treated bamboo fibers were analyzed using a thermogravimetric analyzer (TGA/SDTA 851; Mettler Toledo, Switzerland). About 3 mg of the fibers were heated in a nitrogen atmosphere with a heating rate of 20 °C/min. The temperature range was set between 30 and 550 °C.

Differential scanning calorimetry analysis of the bamboo fibers was conducted with a Perkin-Elmer DSC-821 (USA) device in a nitrogen atmosphere with temperature ranging from 30 to 450 °C. Additionally, the instrument was connected to a cooling system (cooling rate 30 °C/min). Approximately 6 mg of the sample was weighed in aluminium pans, sealed properly, and scanned at a heating rate of 10 °C/min. Dehydration and melting temperature, as well as the enthalpy values, were recorded.

RESULTS AND DISCUSSION

Morphology

Mechanical, chemical, and enzymatic pretreatment may affect the change in size and smoothness of the treated fibers. The FE-SEM images of untreated (raw material), pulp, bleached, and high pressure water-enzyme hydrolysis bamboo fibers (Fig. 1) shed evidence on the changes of the treated bamboo fiber morphology. In light of the information provided by Fig. 1a, the FE-SEM image of raw bamboo fiber exhibited irregularities on the surface. The typically rough surface of raw bamboo fibers might be due to the presence of non-cellulosic materials including lignin, hemicelluloses, and waxy materials (Mustafa *et al*. 2011; Hossain *et al*. 2014). The surface of the fibers appears to be quite uniform and smooth in the FE-SEM image of pulp (Fig. 1b), bleached (Fig. 1c), and pressurized water-enzymatic treatment (Fig. 1d). The average diameter of the raw, pulp, bleached, and pressurized enzymatic treated fibers was found to be 80.86 ± 1.0 to 180.9 ± 2.2 μm, 8.26 ± 0.4 to 17.87 ± 1.2 μm, 7.425 ± 0.5 to 22.07 ± 0.8 μm, and 4.63 ± 0.2 to 7.34 ± 0.6 μm, respectively. The pulping process liberated single fibers from the fiber bundles. Furthermore, the subsequent bleaching treatment minimally reduced the fiber diameter. In the case of the pressurized water-enzyme hydrolysis, the treated cellulosic bamboo fiber morphology was homogenous and smooth with the smallest fiber diameter (Fig. 1d). The results emphasize that the high pressure enzyme hydrolysis is the most effective process to treat fiber in order to remove non-cellulosic components from the raw material.

FT-IR Analysis

Changes in the functional groups of the fibers before and after each treatment can be determined using FT-IR spectroscopy analysis. Fourier transform infrared

spectroscopy is a crucial technique in the evaluation of tracking the changes of chemical structure with the various treatments of the fiber.

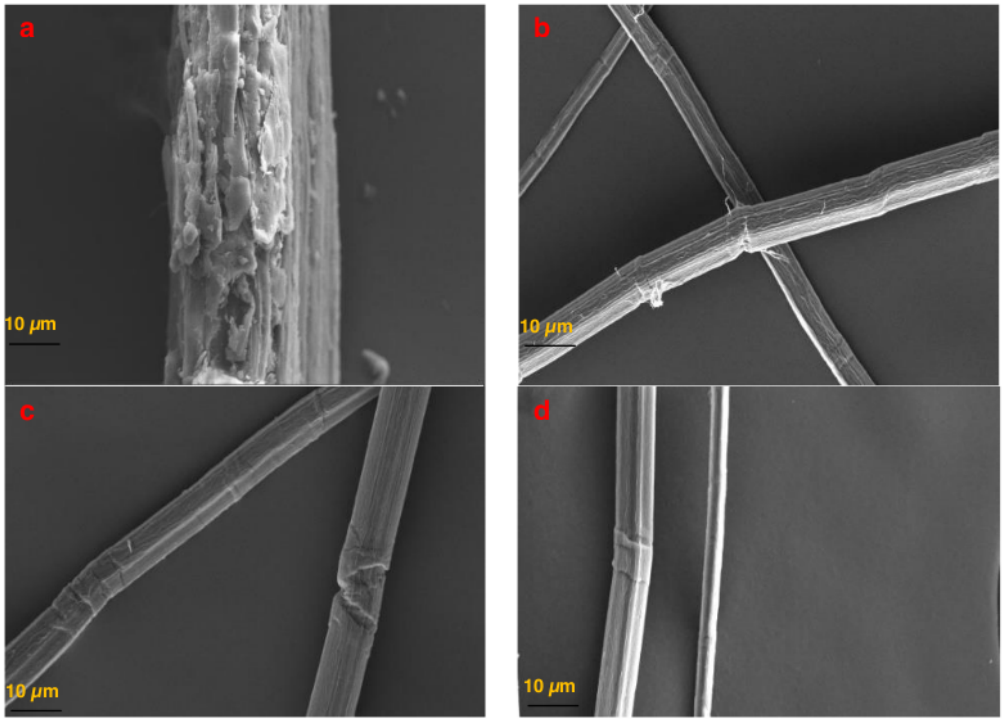

Fig 1. SEM micrographs of (a) raw, (b) pulp, (c) bleached, and (d) high pressure enzyme hydrolysis treated bamboo fibers

Figure 2 shows the FT-IR spectra of raw, pulp, bleached, and pressurized enzyme hydrolysis bamboo fiber. The stretching vibration band between 3000 and 3500 cm^{-1} was attributed to the hydroxyl groups, and the peak at approximately 2900 cm^{-1} was ascribed to C-H stretching (Abdul Khalil *et al*. 2001). The peaks at 1731 cm^{-1}, 1621 cm^{-1}, 1370 cm^{-1}, and 1251 cm^{-1} are related to C=O stretching in the hemicellulose, absorbed water, C-O, C-H groups of the aromatic ring, and C-O stretching in lignin (Jonoobi *et al*. 2009).

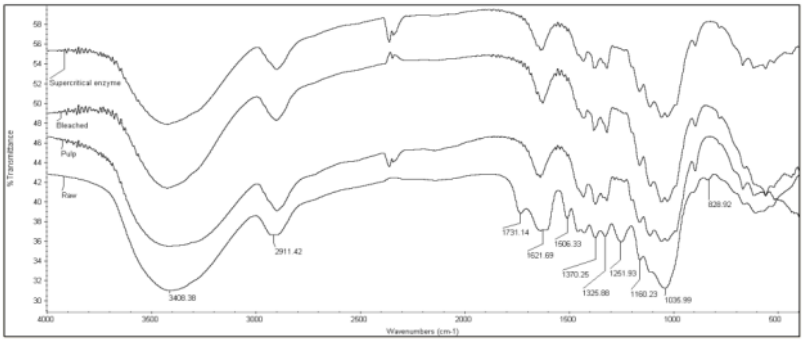

Fig. 2. FT-IR spectra of raw, pulp, bleached, and pressurized water-enzyme hydrolyzed bamboo fibers

The absorption peak at 1506 cm^{-1} was assigned to the aromatic asymmetric stretching of lignin (Chan *et al*. 2012). After pulping, bleaching, and pressurized water treatments caused the peaks at 1731 cm^{-1}, 1506 cm^{-1}, and 1251 cm^{-1} to disappear from the spectra, which confirmed the removal of lignin and hemicellulose using the applied treatments. Furthermore, the O-H and C-H stretching can be seen around 1035 cm^{-1}, and the C-O-C band formed a peak around 1160 cm^{-1} (Jonoobi *et al*. 2009). Finally, the vibrational peak at approximately 828 cm^{-1} was due to the glycosidic bond (Chan *et al*. 2012).

X-ray Diffraction Analysis

X-ray Diffraction (XRD) is the most commonly used method to measure the crystallinity of cellulosic fibers. The diffraction peak pattern in the XRD spectrum reveals the crystalline state of a sample. It has been reported that cellulose is partly crystalline and partly amorphous in its molecular structure (Abdul Khalil *et al*. 2010a; Jonoobi *et al*. 2011). These attributes are due to the cellulose chain that is held closely by mutual H-bonding in the crystalline region and the absence of such organized H-bonding in the amorphous region (Jonoobi *et al*. 2011).

The crystalline values of the cellulosic fiber may affect the physical or chemical approach by influencing the H-bonds in the crystalline region. The crystalline formation of the bamboo cellulosic fiber was determined and compared with the various treatment processes, as presented in Fig. 3.

Results show the native cellulose crystalline structure is due to the formation of peaks at 2θ between 18 and 22.5 °C. It was observed that the crystallinity values differ with each treatment process. The crystallinity values of the raw, pulp, bleached, and pressurized enzyme hydrolysis were 47.91%, 54.34% 60.89%, and 67.97%, respectively. The highest crystallinity value obtained was about 68% for the pressurized enzyme hydrolysis of bamboo fibers, which can attribute to the efficient removal of non-cellulosic components from the raw fiber by the pressurized water-enzyme hydrolysis. However, the increase in the crystallinity observed after pressurized enzyme hydrolysis was probably due to re-arrangement of the disordered fraction of fibers (Jonoobi *et al*. 2011).

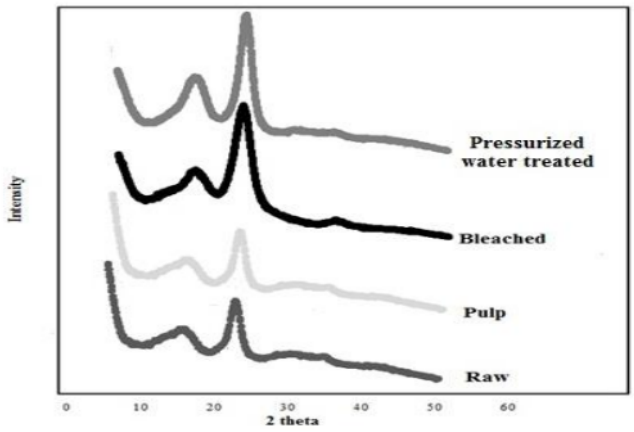

Fig. 3. XRD diffractograms of raw, pulp, bleached, and supercritical treated bamboo fibers

Thermal Properties

Cellulosic materials are known to undergo rapid thermal degradation at low to moderate temperatures. Therefore, the thermogravimetric analysis was performed to evaluate the weight loss, decomposition temperature, and thermal stability of the bamboo fibers throughout each treatment process, as shown in Fig. 4. The thermal characteristics of the fibers, including the initial decomposition temperature (T_{onset}), maximum degradation temperature (T_{max}), and char residue at 550 °C, are summarized in Table 1. Results indicate that raw, pulp, bleached, and pressurized enzyme hydrolysis cellulosic bamboo fiber possessed an initial decomposition below 150 °C, due to evaporation of water. T_{onset} values of the raw bamboo, pulp, bleached, and pressurized enzyme hydrolysis fibers were found to be 308, 330, 337, and 343 °C, respectively. The initial degradation temperature increased after the raw bamboo fibers were treated with pulping, bleaching, and pressurized enzyme hydrolysis processes. The pressurized enzyme hydrolysis bamboo fibers showed the highest T_{onset} values, and similar trends were observed in the case of maximum degradation temperature (T_{max}) values. The T_{max} values were 354, 357, 358, and 369 °C for the raw, pulp, bleached, and pressurized enzyme hydrolysis bamboo fibers, respectively.

The highest maximum degradation temperature (T_{max}) of the pressurized enzyme hydrolysis bamboo fibers reveals the increase in fiber crystallinity during the treatment process. The higher crystallinity leads to greater heat resistance, thus boosting the thermal stability. The amount of residue present at 550 °C was reduced from 23% to 7% for the pressurized enzyme hydrolysis bamboo fibers. A higher amount of residue in the raw fibers directly correlates to the presence of lignin, hemicelluloses, and extractive non cellulosic materials. Degradation of char residue reveals that pulping, bleaching, and pressurizing enzyme hydrolysis treatment of bamboo raw fiber reduces the overall char residue from the raw bamboo fibers (Jonoobi *et al*. 2011).

For the composite application, most of the polymers need to be processed at temperatures above 200 °C. Thus, the bamboo fibers should have high thermal stability in order to use polymer composite materials. The findings of this study show that high pressure enzyme hydrolysis can potentially to be utilized in the production of quality cellulosic bamboo fiber to be used as a reinforcement agent in functional composites applications.

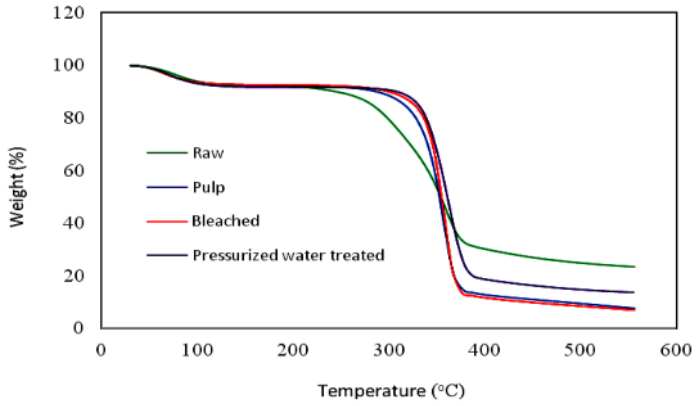

Fig. 4. TGA thermograms of raw, pulp, bleached, and pressurized enzyme hydrolysis bamboo fibers

Table 1. Thermal Properties of Raw, Pulp, Bleached, and Pressurized Water-Enzyme Treated Bamboo Fibers

Sample	T_{Onset} (°C)	T_{max} (°C)	Residue (%)
Raw	308.01	354.26	23.41
Pulp	329.91	356.79	13.72
Bleached	337.09	357.86	7.85
Pressurized water enzyme	342.89	368.07	7.21

CONCLUSIONS

1. A high-pressure enzymatic hydrolysis process was utilized to treat bamboo fiber, and the performance results of this process were compared with that of the pulping and bleaching processes.
2. The FE-SEM analysis revealed that pressurized water-enzyme hydrolysis treated bamboo fibers were homogenous and smooth in structure with the minimal fiber diameter (4.63 to 7.34 μm).
3. The FT-IR analysis showed that the high pressure water enzyme hydrolysis process can be used to effectively remove the non-cellulosic components in raw bamboo fibers including hemicellulose, lignin, and waxy materials.
4. X-ray diffraction analysis shows that the percentage of crystalline formation values differ with the treatment processes. The crystallinity values of the raw, pulp, bleached, and pressurized enzyme hydrolysis treatment of bamboo fiber were 47.91%, 54.34%, 60.89%, and 67.97%, respectively. The highest crystallinity formation for the pressurized water-enzyme hydrolysis for the bamboo fibers can be attributed to the efficient removal of non cellulosic component from the raw fibers.
5. Thermogravimetric analysis revealed that the thermal stability of the bamboo fibers increased with the pulp, bleached, and high pressure water enzyme hydrolysis process. However, the highest T_{max} value (369 °C) was gained for the pressurized enzyme hydrolyzed bamboo fibers.
6. Based on the results, it was found that high pressure enzyme hydrolysis is an effective process for producing cellulose bamboo fiber with elevated crystallinity and higher thermal stability compared to the pulping and bleaching processes. Thus, the high pressure enzyme hydrolysis generates remarkable interest for the utilization in the production quality of cellulosic fiber as a reinforcement agent in functional composite applications.

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