Bioprocessing of Bamboo Materials

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Abstract
Bamboo culms were processed using microorganisms, and fibre bundles were obtained. Bacteria and fungi with xylanase activity were isolated from the bamboo retting system. Chemical composition analysis of the fibre bundles obtained showed that the components are mainly cellulose, hemicelluloses and lignin. An increase in cellulose and hemicelluloses content was detected along with a decrease in lignin content after bio-processing. Environmental Scanning Electronic Microcopy of the fibre bundles (retted) showed great fibre sensibility towards moisture, which could significantly influence mechanical properties. Our results suggested that the bio-processing presented herein contributes to the possible development of a new means of bamboo bio-processing that can be regarded as a primary process to separate fibre bundles from non-cellulosic tissue in the culm.

Key words: *bamboo, retting, fiber bundles, microorganisms.*

Introduction

Bamboo is emerging as an alternative source for textile fibre due to its superior physical and mechanical properties, fast-growing and quick-maturing characters as well as its abundant resources. Previous reports about the extraction of fibres from bamboo primarily comprise mechanical [1 - 3], chemical [4 - 6] and combined mechanical-chemical procedures [7, 8]. Fibres obtained from mechanical treatment contain high amounts of noncellulosic substances (lignin and gummy material) resulting in low quality and a stiff hand feeling, hence this kind of fibre is only applied for household and handicraft articles, as well as being a reinforcement for composite materials. It is well known that cellulose fibres in bamboo are oriented along the bamboo culm and are embedded in a ligneous matrix [7]. An efficient way to remove noncellulosic substances is generally severe alkaline treatment. Due to the extremely tight structure of bamboo compared with other bast materials, mechanical pretreatment is also necessary to loosen the culms, which also has the benefit of the penetration of chemicals.

The possibility of a milder biological process has been studied before [9]. This procedure also allows to obtain a higher amount of fibre bundles compared with mechanical treatment [10] and better fibre properties [11]. A significant amount of literature is available on the application of biotechnology for bast fibre extraction [12], like jute [13, 14], hemp [15, 16], kenaf [17, 18] and flax [19, 20], which provide guidelines for bamboo fibre extraction. Very few reports are available on the isolation of microbes [21-24] as well as on the application of commercial enzymes [9, 11, 25] for bamboo bio-retting.

In this paper, a possible bamboo retting system is introduced and microorganisms isolated from the retting system are analysed for the first time. The characteristics of retted bamboo are also discussed.

Experimental part

Retting process

The raw material was from Moso Bamboo harvested in bamboo gardens in the Jiangxi province, China. Bamboo culms were cut into several segments of 50 cm length in a vertical direction, some of which were further cut into pieces from the cross section. The bamboo node and epidermis were not removed. Bamboo segments and pieces were cleaned with flowing water prior to retting. Retting was carried out through the fermentation of bamboo culms soaked in water at room temperature for 2 months. The starting pH was between 6.8 and 7.0. During all exposures, the pH presented only minor variations from the starting point, ranging from 6.4 to 6.7.

Screening and identification of the microorganism in the retting system

Microorganism screening

To collect a wider range of microbes, different retting liquids were collected and investigated, including liquors from retting with distilled water or tap water

Table 1. *Medium formula.*

Medium	Concentration, g/dm^3
Enrichment medium (liquid xylan medium) for bacteria	xylan - 5, $(NH_4)_2SO_4$ - 0.5, yeast extract - 0.5, $MgSO_4 \cdot 7H_2O$ - 0.3, K_2SO_4 - 0.1, KH_2PO_4 - 1.0, $CaCl_2 \cdot 2H_2O$ - 0.2, trace element solution 0.5 ml, tap water, pH 7
Selective medium (solid xylan medium) for bacteria	xylan - 5, $(NH_4)_2SO_4$ - 0.5, yeast extract - 0.5, $MgSO_4 \cdot 7H_2O$ - 0.3, K_2SO_4 - 0.1, KH_2PO_4 - 1.0, $CaCl_2 \cdot 2H_2O$ - 0.2, trace element solution - 0.5 ml, agar - 18, tap water, pH 7
Enrichment medium (liquid xylan medium) for fungi	xylan - 5, $(NH_4)_2SO_4$ - 2, yeast extract - 2, $MgSO_4 \cdot 7H_2O$ - 0.3, K_2SO_4 - 0.1, KH_2PO_4 - 1.0, $CaCl_2 \cdot 2H_2O$ - 0.2, trace element solution 0.5 ml, tap water, pH 5.5
Selective medium (solid xylan medium) for fungi	xylan - 5, $(NH_4)_2SO_4$ - 2, yeast extract - 2, $MgSO_4 \cdot 7H_2O$ - 0.3, K_2SO_4 - 0.1, KH_2PO_4 - 1.0, $CaCl_2 \cdot 2H_2O$ - 0.2, trace element solution 0.5 ml, agar - 18, tap water, pH 5.5
Bamboo medium	bamboo powders - 20, $(NH_4)_2SO_4$ - 2.5, K_2HPO_4 - 0.5, $MgSO_4$ - 1, agar 20 g, pH nature
Trace element solutions	$FeSO_4 \cdot 7H_2O$ - 5, $MnCl_2 \cdot 4H_2O$ - 3, $ZnSO_4 \cdot 7H_2O$ - 1.4, H_3BO_3 - 3, $CoCl_2 \cdot 7H_2O$ - 2, $NiCl_2 \cdot 6H_2O$ - 2, $CuSO_4 \cdot 5H_2O$ - 1, H_2SO_4 95% 4 ml, distilled water

covered with plastic paper (loose) at the top (aerobic); retting with tap water covered with plastic paper (tight) at the top (anaerobic); retting bamboo culms of different ages, and retting bamboo culms from different regions.

The media composition for the isolation of the microorganism is listed in ***Table 1,*** with the aim of screening xylanase-producing strains with minor modification according to previous studies [26 - 28]. 1ml of retting liquid was incubated in 100 ml of enrichment liquid for bacteria at 37 °C for 24 h and for fungi at 28 °C for 3 - 4 days, respectively, in order to increase the growth of the target microorganism. After that, 0.1 ml of enriched retting liquid was properly diluted with sterile NaCl solution (0.85%), then plated on petri dishes with a selected xylan medium and finally cultivated at 37 °C for 2 - 4 days and 28 °C for 4 - 7 days for bacteria and fungi, respectively, with the purpose of obtaining individual ideal strains. Single colonies were picked, transferred to the new xylan plates (selective medium) again and then incubated under the same conditions as mentioned above. Those colonies were also cultivated on petri dishes with a bamboo medium to check the growth possibility in the bamboo system. Bacteria and fungi grown well on both xylan and bamboo mediums were kept and stored in a Nutrient Broth (NB) and Potato-Dextrose Agar (PDA) sloped tubes at 4 °C, respectively.

Identification of microorganisms

Microorganisms isolated from the retting liquid were identified with 16S ribosomal DNA (rDNA)-based techniques. Bacteria were incubated in a fresh NB liquid medium at 37 °C, 150 r.p.m. for 18 h, and then the DNA was extracted by kit from BioDev using a DNA extraction reagent according to the instructions of the manufacturer. The 16S rRNA gene was amplified through polymerase chain reactions (PCR) carried out in a Bio-Rad iCycler PCR System by use of the forward primer BSF8/20 (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse primer BSR1541/20 (5'-AAG GAG GTG ATC CAG CCG CA-3') [29]. The PCR was performed in a 50 µl reaction volume containing 5 µl of a 10×PCR Buffer (TaKaRa Mg^{2+} Plus), 4 µl of a deoxynucleoside triphosphate (dNTP) Mixture (TaKaRa 2.5 mM each), 0.25 µl of Taq DNA Polymerase (TaKaRa 5 U/µL), 2 µl of each primer (50 pmol), 2 µl of each DNA extract (around 2.5 ng), and 34.75 µl of Sterile deionized water. The cycling profiles were as follows: 5 min pre-denaturing at 94 °C, 1 min denaturation at 94 °C for 35 cycles, 30 s annealing at 53 °C, 90 s extension at 72 °C and a post-run of 10 min extension at 72 °C [30].

For fungi, 50 - 100 mg of dried mycelium was used for DNA extraction performed according to the method described by Aljanabi and Martinez [31]. Fungal DNA was amplified using the primer ITS1f (5'-TCC GTA GGT GAA CCT GCG G-3'), which hybridises at the end of 18S rDNA, and primer ITS4rp (5'-TCC TCC GCT TAT TGA TAT GC-3'), hybridising at the beginning of 28S rDNA [32]. PCR reactions were performed in volumes of 40 µl containing 8.0 µl of Tag-& Go (MP, 5×C), 1.2 µl of magnesium chloride solution (Fluka, 50 mM), 1.6 µl of each primer (Biomers.net, 10pmol/µl), 25.6 µl of sterile deionised water and 2.0 µl of each DNA extract. The reaction consisted of an initial denaturation at 95 °C for 5 min, followed by 37 cycles of 95 °C for 30 s, 54 °C for 35 s, and 72 °C for 40 s, and then completed by a 10-min extension at 72 °C. All the PCR products (bacteria and fungus) were purified with GENECLEAN® Turbo Kit before sending to sequence. The results of the sequences were compared with those available at the GenBank of the National Center for Biotechnology Information by means of the Basic Local Alignment Search Tool (BLAST) to determine phylogenetic affiliations.

Characterisation of retted bamboo

Morphology changes observation

Morphological characterisation of bamboo culms during retting was observed by visual inspection and by means of an optical microscope, Nikon Eclipse 400, in the transmission mode.

Spectroscopic analysis

The components of powdered fibre bundles (retted) as well as the substance released from bamboo culms during the retting process (i.e. retting-removed substrates) were analysed by attenuated total reflectance Fourier transform infrared (ATR-FITR) spectroscopy. Samples were dried in an oven at 100 °C for 3 h prior to testing. Infrared spectra were taken on a Nicolet 5700 spectrometer (Thermo, USA) equipped with an ATR unit, using a diamond crystal to measure the reflectance from the samples. After cleaning the crystal area and collecting the background, a suitable number of samples were placed on the crystal area. Spectra were acquired at a resolution of 4.0 cm^{-1} in the wavelength range from 4000 to 400 cm^{-1}.

Chemical analysis

The chemical composition of the original bamboo culm (without outmost layer) and retted bamboo (fibre bundles) was made according to Chinese Standard GB5889-86 (the method for quantitative analysis of bast fibre chemical components). There was a minor modification made for the organic solvent extractive content test. Toluene solution was used instead of benzene solution. Each test was performed in duplicate, the average results of which are shown.

Sensitivity of environmental humidity / moisture

Water uptake

Bamboo raw material (bamboo pieces) and retted bamboo (fibre bundles) were soaked in water overnight to wet completely. Afterwards, water on the surface of the fibre bundles was gently removed by filter papers and the weight was recorded as G_0. Then the fibre bundles were dried in an oven for 3 h at 100 °C and weighted directly in the oven to get the completely dry weight of G_1. The water uptake was assessed using the following formula:

$$\text{Water uptake} = [(G_0 - G_1)/G_1] \times 100\%$$

where,

G_0 - completely wet weight of sample (bamboo raw material or retted bamboo),

G_1 - completely dry weight of sample (bamboo raw material or retted bamboo).

Wettability

Wettability is estimated by using the contact angle test. The liquid-solid contact angle was followed by means of a JY-82 Contact Angle Meter (HARKE) with respect to characterising water repellency on the surface of the oven-dried (45 °C, 1 h) fibre bundles (obtained by retting). The bundles were conditioned in the lab at a constant temperature of 20 ± 2 °C and relative humidity of 65 ± 2%, according to Textiles-Standard atmospheres for conditioning and testing (ISO 139:2005 and GB/T 6529-2008), for 2 h prior to the contact angle testing.

Moisture desorption and absorption

A certain amount of fibre bundles (obtained by retting) were dried in the oven at 45 °C for 0.5 - 1 h and stored in a desiccator for moisture absorption testing. Meanwhile, some bundles were soaked in water for 48 h for moisture desorption testing, prior to which water on the surface of wet samples was removed by filter paper. The weight of samples was recorded every 5 min by electronic balance. Experiments were carried out in the lab with Textiles-Standard atmospheres as indicated above. The weight recorded at 5 min was regarded as the reference and the weight changes were compared with this value.

The deformation behaviour of the bamboo fibre bundles (obtained by retting) was assessed in relation to different humidities

The deformation behaviour of a single elementary bamboo fibre bundle (obtained by retting) was investigated by environmental scanning electron microscopy (ESEM, Philips XL30) equipped with a Peltier cooling stage. Temperature adjustments for the sample were made using a Peltier stage. Before observation, the temperature of the Peltier cooling stage was set to 0 °C. Samples were cut from the cross section and placed onto the stage vertically by means of electrically conductive adhesive transfer tape. The pressure inside the specimen chamber was varied from 6.1 to 0 torr in steps of 0.1 torr per 2 min for the purpose of varying the relative humidity from 100% to 0%. It was unnecessary to coat the sample for this analysis. Observations of the behaviour of the sample (cross-section) were made at each point of interest, with images acquired at a temperature of 5 °C.

Softness of fibre bundles obtained by retting

The softness of fibre bundles (obtained by retting) is mainly responsible for its twisting ability. Therefore, twist testing was carried out to determine the softness indirectly. Both dry and wet bamboo fibre bundles (obtained by retting) were tested on a Y331A Yarn Twist Counter Tester with a clamp distance of 10 cm and pre-tension of 20 cN . Each piece of the bundles was twisted until it broke, And the number of twists was counted directly.

Single fibre bundle (retted) tensile test

The single fibre bundle (retted) tensile test was used to evaluate the strength properties of the wet and dry samples. The test was performed on a Universal Material Testing Machine (Model H5K-S, Hounsfield, USA) equipped with a HT 400 Pneumatic Grip Controller. Tests were performed at 20 °C and 65% relative humidity with a gauge length of 10 cm and strain rate of 50 mm/min. 30 specimens were successfully tested for each sample. Samples were conditioned for 24 h under the standard atmosphere mentioned above prior to testing.

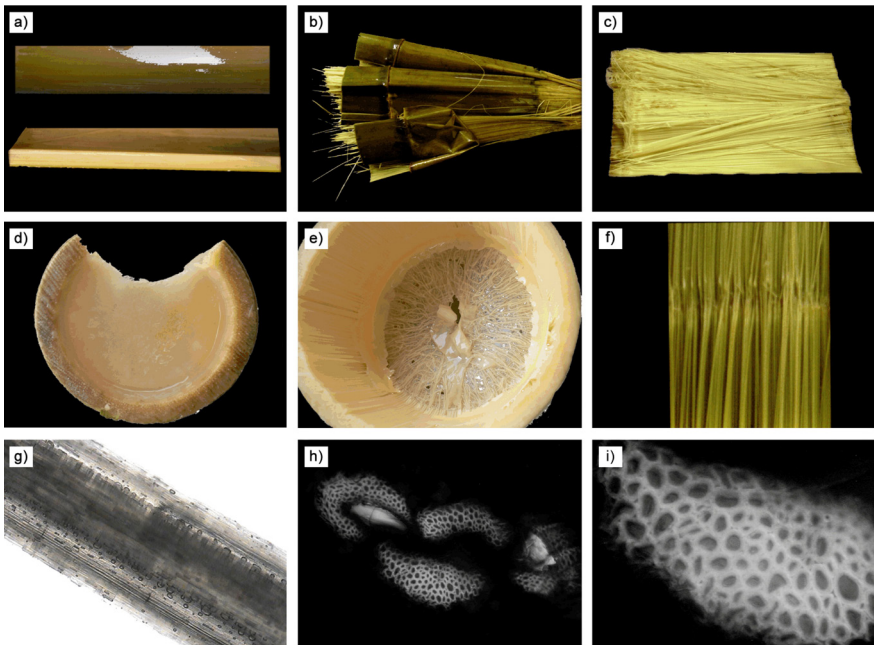

Figure 1. *Morphology of bamboo before and after aerobic retting for 2 months: a - original bamboo culm; b - retted bamboo culm, c - fibre bundles obtained by retting, d - original bamboo septum, e - retted bamboo septum, f - fibre bundles in the node obtained by retting, g - vertical section of fibre bundles obtained by retting (×40), h - cross section of fibre bundles obtained by retting (×40), i - cross section of fibre bundles obtained by retting (×400).*

Results and discussion

Observation of morphology changes during the retting process

In this study, bamboo culms were incubated in water to investigate the retting effect on different parts of bamboo. The morphology changes of bamboo culms before and after retting were inspected visually. The different kinds of retting methods (anaerobic or aerobic retting) led to the same phenomenon change i.e. the separation of bundles from the culm. ***Figure 1***. presents the general morphology changes of bamboo culms during the processing. After retting, the three layers of bamboo culms were obviously separated from each other since the materials (mainly hemicellulose) binding the bundles together had been degraded by the microbial enzymes. Therefore, the original integrating unit of the bamboo culms could be separated into epidermis (the outmost layer, ***Figure 1.b***), bundles of small fibres (parallel to each other, ***Figure 1.c***) as well as into the degraded innermost layer, wrapped by sclerenchyma cells. It is obvious that the epidermis of bamboo is difficult to degrade by microbial retting due to its extraordinarily tight structure, based on axially elongated cells, shorter cork and silica cells. It contains a high amount of silica (silicon dioxide) and a waxy layer called cutin, which makes the outmost layer compact and strong. Thus, this structure is responsible for water blockage and tissue protection [33]. Bamboo culms are particularly strong at their nodes. An interesting result from the bamboo node observation (***Figure 1.d, 1.e*** and ***1.f***) reveals that fibre bundles are not broken or cut by the septum. In fact, fibre bundles twist with each other at the node area (***Figure 1.e***). When the inner materials are degraded, fibre bundles could be untwisted. This result agrees with the fact that cells are transversely inter-connected at the node and axially oriented at the internodes. As is observed from the morphology of the vertical- (***Figure 1.g***) and cross- (***Figure 1.h*** and ***i***) section, a single fibre bundle obtained by retting consists of many single fibres in a hollow structure. Our results suggest that fibre bundles of any length could be obtained, which is beneficial for downstream processing to meet the requirements for spinning. Since monofilament bamboo fibre is only

Table 2. *Identification of isolated microorganisms based on the 16S ribosomal RNA.*

Strain No.	Closest relative microorganism	Similarity, %	Accession number
B1	*Enterobacter* sp.	100	FJ593001
B2	*Bacillus* sp.	97	DQ314537
B3	*Pantoea* sp.	99	EU302841
B4	*Pseudoxanthomonas* sp.	97	EF219047
B5	*Psychrobacter* sp.	100	AJ551129
B6	*Bacillus* sp.	99	FJ528074
B7	*Bacillus* sp.	97	EU584544
B8	*Comamonas* sp.	97	FM877975
B9	*Acinetobacter* sp.	99	FJ607348
B10	*Bacillus* sp.	98	EU584552
B11	*Acinetobacter* sp.	97	FJ607348
B12	*Bacillus subtilis* subsp.	95	AM237380.1
B13	*Enterobacter* sp.	95	DQ855282.1
B14	*Streptomyces* sp.	97	EU257256.1
B15	*Paenibacillus* sp.	96	EF203083.1
F1	*Acremonium strictum genogroup III*	99	AY138846.1
F2	*Fusarium oxysporum* f.	99	EF590328.1
F3	*Penicillium* sp.	99	EU301633.1
F4	*Penicillium* sp.	99	EU301633.1
F5	*Emericella* sp.	99	AB249018.1
F6	*Emericella* sp.	99	AB249018.1

1 - 2 mm in length [34] and cannot be spun, it is the essential to control the degree of bamboo degumming and leave some non-cellulosic substances to stick single fibres together, thus achieving the length and fineness required for textile application. Complete degumming should be avoided in bamboo fibre processing. In this case, the great advantage of bioretting over mechanical scratching or steam explosion or traditional chemical processes is that it is a mild and controllable process, and the long bundles obtained could be further separated, gaining the possibility of meeting the textile requirements.

Isolated microorganisms

Microbes isolated from the retting system, based on 16S rDNA sequence analysis, comprised *Bacillus* sp., *Acinetobacter* sp., *Pseudoxanthomonas* sp., *Comamonas* sp., *Psychrobacter sp., Pantoea* sp., *Streptomyces* sp., *Paenibacillus* sp. and fungi consisting of *Acremonium strictum, Fusarium oxysporum* f., *Penicillium* sp., *Emericella* sp. (shown in ***Table 2***). The accession number corresponds to the individual sequence record defined in the GenBank®. With the sequence result and additional comparison with the seqence in the GenBank database, we can get a clear idea of the strain species. For example, in ***Table 2***, B1 to B15 represent isolated bacteria strains, and F1 to F6 - isolated fungi strains. B2, B6, B7 have high similarity with the sequences in the NCBI GenBank database under the accession numbers of DQ314537, FJ528074 and EU584544, respectively. Moreover the sequences with accession numbers of DQ314537, FJ528074 and EU584544 are defined as bacillus sp., therefore, the B2, B6 and B7 strains are identified as *Bacillus* sp. Species of the *Bacillus* genus were dominant among the bacteria isolated from the retting system. High xylanase extracts of *Bacillus* have been reported as acting in acidic [35] and alkaline [36, 37] conditions. Clearing zones were observed on the xylan plates for fungal strains (results not shown here), indicating the degradation of xylan in the medium (results not shown here). Overall the microbes from the retting system analysed seem to produce high xylanolitic activity and are therefore well suited to grow over bamboo materials, which contain a high content of hemicellulose (25%), of which 90% is xylan [38, 39].

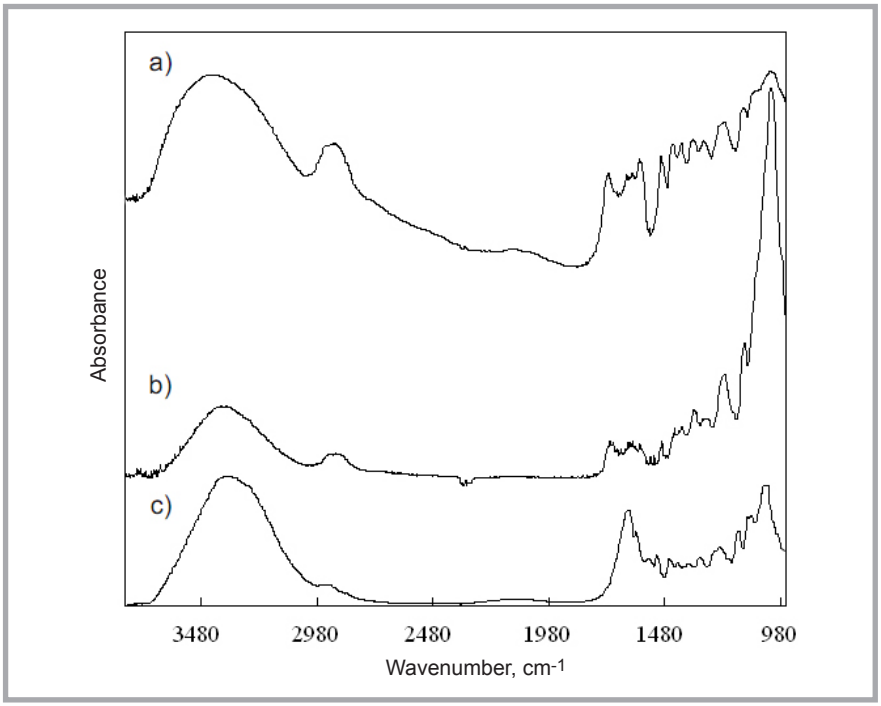

Figure 2. *FTIR-ATR spectra: a - original bamboo, b - retted bamboo (fibre bundles), c - substances removed during retting.*

Chemical composition analysis

An analysis of the residue removed from the bamboo pieces during the retting process was performed by FTIR (***Figure 2***), where a comparison was made with retted bamboo (fibre bundles) and original bamboo (bamboo pieces). Differences are found in the range of 1600 cm^{-1} to 1450 cm^{-1}. The bands at 1604 cm^{-1}, 1548 cm^{-1}, 1461 cm^{-1} and 1454 cm^{-1} are related to skeleton stretching vibration of the aromatic rings and C-H deformation [40 - 43], which mainly concern the lignin in bamboo. The relative intensity of the bands appears in the original bamboo (***Figure 2.a***), and the retting-removed substances (***Figure 2.c***) are much stronger than those in the retted bamboo (fibre bundles) (***Figure 2.b***). A band at 1245 cm^{-1} originating from C-O-C asymmetric stretching vibration could be observed in the retted bamboo (fibre bundles) spectrum, which is the only clear indication of the existence of lignin.

However, the intensity is lower compared with other bands in the spectrum. Considering the fact that the FTIR spectroscopy of cellulose has the strongest and characteristic spectrum bands in the range of 1070 - 1040 cm^{-1}, the specific intense bands at 1051 cm^{-1} and 1043 cm^{-1} denote the presence of celluloses in both samples. In particular, this peak intensity in retted bamboo (fibre bundles) is evidently stronger compared with others. Thus, retted bamboo (fibre bundles) has a higher concentration of cellulose. In addition, specific bands related to cellulose could be found at 1425 cm^{-1}, 1322 cm^{-1}, 1164 cm^{-1} and 910-885 cm^{-1}, which correspond to the scissor oscillation of CH_2 groups, O-H blendig (in-plane), antisymmetric bridge C-O-C stretching vibration and C-H deformation, respectively. Moreover, bands at 2919 cm^{-1}, 1730 cm^{-1}, 1385-1355 cm^{-1}, 1170-1142 cm^{-1}, 1122 cm^{-1}, 1106 cm^{-1}, 1037 cm^{-1}, 992 cm^{-1} and 910-885 cm^{-1} possibly originate from both the cellulose and hemicellulose in bamboo. These observations seem to indicate that the retting-removed substances (i.e. the substance released from bamboo culms during the retting process) are mainly composed of lignin, hemicelluloses and a certain amount of celluloses. The removal of these substances could result in the loosening of the bamboo culm, further leading to the separation of fibre bundles. Since the main components (cellulose, hemicellulose and lignin) in the bamboo culm interpenetrate each other and form acomplex network, part of the cellulose would be removed with the removal of hemicelluloses and lignin. Therefore, bands originating from cellulose could be also detected in the substances removed (***Figure 2.c***).

A more detailed component analysis of raw and retted bamboo was carried out using a chemical method to get quantative information. As shown in ***Table 3***, it is clear that bamboo raw material (bamboo pieces) contains a relatively lower amount of cellulose and a higher amount of lignin and hemicellulose when compared with traditional fibrous materials like kneaf. The retting did not change the relative composition significantly. Hot-water extractives, organic solvent extractives and lignin seem to be the major compounds removed from bamboo culm, as indicated in ***Table 3***. An interesting discovery was that pectin "appeared" during retting, which was due to a marginal amount of pectin (0.2%) detected in the bamboo fibre bundles (retted), while in the unretted bamboo (bamboo pieces), pectin was undetectable. One possible explanation is that pectin was not accessible in the unretted bamboo due to bamboo's naturally tight and compact structure, and to the fact that its amount was relatively lower compared with other components. Retting helps loosen the structure of bamboo, therefore, pectin might expose to the chemicals to detection. In addition, the organic solvent extractives were almost removed throughout the retting process, indicating no obvious amount of waxy remaining on the fibre bundles, which might be beneficial for further enzymatic treatment. Although about 20% of the bundles are lignin, some peaks of the lignin did not exist in the FTIR spectra. This might be the limitation of the testing methods, since FTIR is more suitable and accurate for the chemical analysis of pure samples. The multi-components in the sample can cause more peak generation, making it difficult to avoid the peaks overlapping. Thus a chemical analysis – FTIR comparison would be more informative for the fibres. Based on the test above, it can be stated that hemicelluloses and lignin are the main compounds on the outer wall of the fibre bundles since they can be removed by retting.

Table 3. *Chemical analysis of bamboo and kenaf in %.*

Samples	Cellulose	Hemicellulose	Lignin	Pectin	Organic solvent extractives	Hot-water extractives
Bamboo pieces	39.4	26.5	23.2	0.0	4.8	6.0
Bamboo fibre bundles (retted)	42.3	35.6	19.4	0.2	0.0	2.6
Unretted Kenaf [18]	53.2	14.3	8.2	8.7	0.5	15.0

Sensibility of moisture

Bamboo culm is a hygroscopic material and therefore sensible to the relative humidity of the surrounding environment. In contrast to wood which shrinks or swells when the moisture is below the fibre saturation point (FSP), bamboo changes dimensions as soon as it starts to lose moisture [44].

The water uptake of retted bamboo (fibre bundles) is extremely high. Results show that the weight of completely wet fibre bundles could achieve almost 4.64 times as much as that of dry bundles, while the wet bamboo raw materials was around 2.56 times as much as that of dry bamboo in weight. It seems that bamboo bundles uptake water more easily, which could be attributed to their different structures. Retting makes the structure of bamboo material looser, thus, more water can be carried.

The contact angle is an indicator of the affinity of a liquid for a solid. The shape of the liquid drop on a solid surface is related to the magnitude of the cohesion forces acting between the three planes: solid, liquid, and gas [45]. A contact angle of around 4° was obtained for the dry sample. As water droplets exhibit contact angles of 0° to 30° on most highly hydrophilic surfaces [46], this result indicates that bamboo fibre bundles obtained by retting have an excellent wettability of water and belong to the group of highly hydrophilic materials.

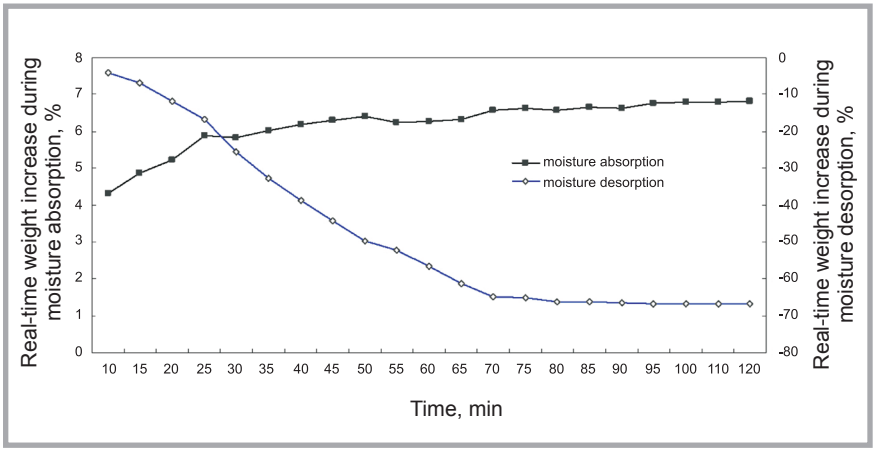

Figure 3. *Real-time weight change of fibre bundles under the condition of moisture desorption and adsorption (the weight recorded at 5 min was used as a standard).*

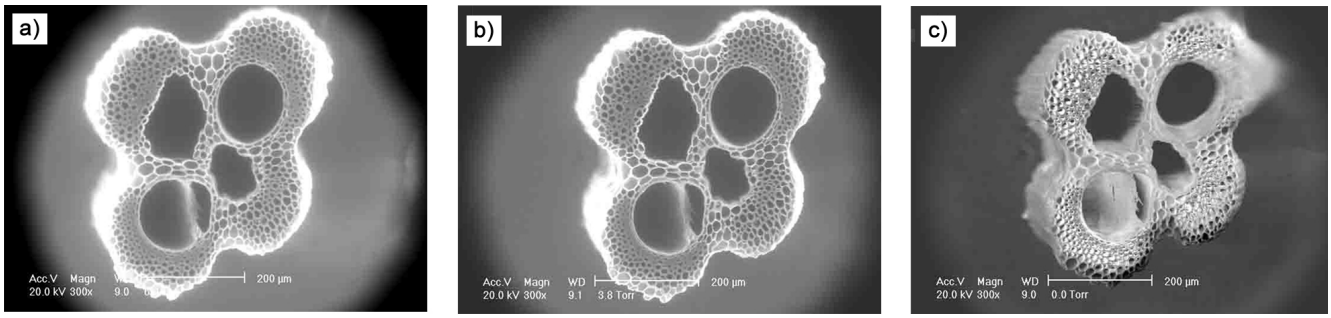

Figure 4. *Moisture sensibility of retted bamboo (fibre bundles) recorded by an Environmental Scanning Electronic Microscope: humidity changes from 100% to 0% (a→c).*

The real-time weight change of retted bamboo (fibre bundles) was recorded under the condition of moisture desorption and absorption. As presented in ***Figure 3*** (see page 18), it takes 40 min for a dry sample to absorb water and 80 min for a completely wet sample to desorb water and arrive at a stable stage in which weight loss or gain is not obvious. Bamboo fibre bundles obtained by retting are quick to achieve stable weight in different situations, which proves their great sensibility to environment humidity.

It is generally agreed that bamboo will start to shrink both in the wall thickness and diameter as soon as it starts to lose moisture [44, 47]. However, the same phenomenon could be observed when exposing retted bamboo (fibre bundles) to moisture fluctuations. The dynamic deformation of retted bamboo (fibre bundles) with a decrease in humidity in the testing chamber was recorded by Environmental Scanning Electron microscopy (ESEM). The observation of the sample during a controlled decrease in the relative humidity - from 100% to 0% - of the ESEM chamber is shown in ***Figure 4***. It is possible to see that the fibre bundles (obtained by retting) undergo an important morphological change during the drying process. The morphology change at the initial stage is not so obvious. The elapsed time from 813 Pa (***Figure 4.a***) to 507 Pa (***Figure 4.b***) (less than 1 h) was not long enough to produce any detectable contraction of the fibre bundles. With a continuous decrease in the humidity of the chamber, the fibre bundles started to collapse. The difference in the morphology recorded under a humidity of 100% (813 Pa ***Figure 4.a***) and 0% (0 Pa ***Figure 4.c***) is visible. Bamboo is subjected to short-term and long-term variation in the surrounding relative humidity and temperature. Consequently, this material always undergoes at least small changes in moisture content, due to the fluctuation of the surrounding environment. The sensibility of humidity is a key point for further separation of fibre bundles and extraction of thinner fibre bundles from retted semi-products.

Softness and tensile properties

The moisture content of retted bamboo (fibre bundles) influences its softness and tensile properties significantly. The softness of fibre bundles obtained by retting is evaluated by calculating twist numbers which could make a 10 cm fibre bundle break. The results demonstrate that the number of twists for a dry sample is generally lower than that for wet samples. The average value of twist numbers from ten tests is 24.22 and 27.44 for a dry and wet sample, respectively. The higher the content of moisture, the softer the hand feeling. Thus, a higher number of twists would be added on the bundles. The twist unevenness (CV value) for a wet sample from ten tests is 33.09%, which is much higher than the 6.67% for a dry sample, due to the different moisture contents in the fibre bundles. Since the ability of water uptake of the different bundles differed from each other, unevenness increased.

The mechanical properties of retted bamboo (fibre bundles) are expressed as the ratio value of tensile and elongation compared with the lowest value achieved in tensile and elongation tests, respectively, since it is difficult to determine the diameter of every fibre bundle with a hollow structure. Results indicate that the tensile ration of dry bamboo bundles is higher than that of wet bamboo bundles, while the elongation rate is lower than that of wet bamboo bundles. The tensile ratio for a dry sample was 1.164 times as much as that for wet samples, while the elongation ratio for a dry sample was 0.955 times as much as that for wet samples. The results obtained correspond with the previous study [48], proving that water absorption causes a reduction in the strength and rigidity of bamboo.

Summary

This paper describes the effect of the bamboo bio-retting process on the characteristics of retted bamboo (fibre bundles). The results demonstrate that fibre bundles could be separated from a bamboo culm by retting. Hemicelluloses and lignin, responsible for keeping the bundles together, were degraded by microorganisms during the retting process. Microbes with a potential xylanase-producing ability were isolated from the retting liquid. *Bacillus* sp. was found to be the dominant species among the bacteria screened. However, future investigations should identify the most effective strains (e.g. xylanase, pectinase, cellulase producers), optimise the best enzyme activities suitable for bamboo retting, apply multi-microbes and produce enzymes for bamboo degumming.

The investigation of the major properties of bio-retted bamboo (fibre bundles) shows the potential of a bio-based retted system for bamboo. Retting makes the structure of bamboo looser, gaining a great ability to uptake more water, which benefits further enzymatic or mild chemical treatment. Noncellulosic substances like hemicellulose and lignin could be removed during retting. However, retted bamboo (fibre bundles) contains 20% of lignin and 35% of hemicellulose, which still need to be removed for further separation of the bundles. Since no significant variations in the overall chemical compositions after bio-rettingwere verified, it seems to show that the bio-retting of bamboo is very gentle treatment. Seeking a controllable way to treat bamboo and aiming to obtain fibres step by step are the key points in bamboo degumming. Bio-retting can be the effective pretreatment in bamboo processing.

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