ANALYSIS OF THE CHEMICAL COMPOSITION AND MORPHOLOGICAL STRUCTURE OF BANANA PSEUDO-STEM

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An analysis of the chemical composition and anatomical structure of banana pseudo-stem was carried out using Light Microscopy (LM), Scanning Electron Microscopy (SEM), and Confocal Laser Scanning Microscopy (CLSM). The chemical analysis indicated there is a high holocellulose content and low lignin content in banana pseudo-stem compared with some other non-wood fiber resources. These results demonstrate that the banana pseudo-stem has potential value for pulping. In addition, we report for the first time from using LM and CLSM that banana stems possess a structure involving helicoidal fibers separated by barrier films.

Keywords: Banana pseudo-stem; Chemical analysis; Scanning Electron Microscopy (SEM); Light Microscopy (LM); Confocal Laser Scanning Microscopy (CLSM)

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INTRODUCTION

In recent years, people have placed a high emphasis on forest preservation and rational use of forestry and agricultural residues. This trend is mainly motivated and accelerated by the dilemma of an ever-increasing consumption of wood fiber-based products relative to dwindling wood resources. Furthermore, the application of cellulose fibers has many advantages: it is environmentally sound, recyclable, and low in cost. In 2006, the annual global production of lignocellulosic fibers from crops was about 4 billion tons, of which 60% came from agriculture and 40% from the forest. In comparison, the annual world production of steel was around 0.7 billion tons, and plastic was about 0.1 billion tons (Justiz-Smith et al. 2008).

In general, banana pseudo-stem is an abundant natural resource in subtropical and tropical regions and has potential for providing profitable products such as manure (Ultra et al. 2005) and feed (Ulloa et al. 2004), which call for practical techniques and processes to exploit this natural resource. In South China, the production of banana has significant economical importance. After harvesting banana bunches from the trees over a tract of land, a large amount of waste biomass remains, because each banana plant cannot be used for the next harvest. These bare pseudo-stems are normally felled and usually abandoned in the soil plantation to become organic waste and cause environmental pollution. Therefore, exploitation of the waste banana pseudo-stems will be significantly beneficial to the environment and bring additional profits to farmers.

It has been reported (Justiz-Smith et al. 2008) that the fiber of banana pseudo-stem has a high Young's modulus and water absorption capacity. Moreover, the banana pseudo-stem shows satisfactory physical properties, such as relatively high tensile strength and stiffness, which indicate its prospect as a promising fiber material. The application of natural fibers including banana pseudo-stem has been proved promising in various technical fields, such as replacing synthetic fibers as reinforcement in various composites used in automobile parts (Pothan and Thomas (2003). Zainudin (2009) studied the thermal behavior of banana pseudo-stem (BPS)-filled unplastisized polyvinyl chloride (UPVC) composites. From the study, the thermal stability of the composites was found to be higher than that of BPS fiber and the UPVC matrix. Savastano (2004) studied the application of natural fiberous materials, including banana stem, as reinforcement in cement product. These results demonstrated that these narrow and recycled fibers possessed irregular surfaces and low coarseness; hence, they can be used as cement reinforcement. Additionally, the fiber of banana pseudo-stem has potential for use in absorbents because of its strong water absorption property. Anirudhan (2006) and Noeline (2005) studied new absorbent systems containing banana pseudo-stem. These absorbent systems, which can remove phosphate from wastewater, exhibit high absorption potential and satisfactory recyclability.

Some other valuable products can also be obtained from banana pseudo-stem. For example, the high content of cellulose in banana pseudo-stem has promising potential as a source of man-modified polysaccharide. Adinugraha (2005) studied the synthesis of sodium carboxymethylcellulsoe (CMC) from Cavendish banana pseudo-stem and succeeded in establishing a novel method to utilize Cavendish banana pseudo-stem for preparing sodium CMC. Additionally, Wuyts (2006) succeeded in extracting ployphenol oxidase (PPO), which is a copper-containing enzyme with molecular oxygen as co-substrate.

People have long studied the application of banana pseudo-stem in the pulping and papermaking industry. Guha (1960) studied the kraft pulping process of banana pseudo-stem. Subrahmanyan (1963) drew the conclusion that the fiber of banana pseudo-stem is higher in strength properties than areca husk by comparing their soda pulps. Heikal (1976) developed the nitric acid pulping process of banana pseudo-stem. Dhake (1983) succeeded in producing high-yield chemi-mecahnical pulp of banana pseudo-stem with pretreatment of NaOH. Cordeiro (2003) determined parts of the chemical compositions of banana pseudo-stem and developed the soda-AQ pulping process of banana pseudo-stem. Jahan (2007) studied the formic acid pulping and Totally Chlorine Free (TCF) bleaching of banana pseudo-stem. Manimaran (2007) studied the biobleaching of banana pseudo-stem with xylanase. Cordeiro studied the microstructure of the banana pseudo-stem fiber bundles (2005) and poly-based composites (2006) with SEM and TEM. In this paper, more detailed morphological information of banana stem including pseudo-stem and pith is provided by CLSM observation for the first time.

Further study on the chemical composition and microstructure of banana pseudo-stem will be beneficial to the novel applications of this valuable resource. Therefore, the purpose of this paper is to determine the chemical composition and get detailed morphological information of banana pseudo-stem with variety of measurements.

EXPERIMENTAL

Materials and Chemical Analysis

The banana pseudo-stems were harvested from a banana plantation in Gaozhou (Guangdong, China). The banana pseudo-stem, which was separated from banana bunch and foliage, was about 30cm in diameter and 70cm in height. The layered outer bark can be separated from the pseudo-stem into several blocks by hand. Some samples deprived of pith were air-dried and ground, and the fractions that passed a 40 mesh screen were collected and preserved for chemical analysis. The samples were first submitted to soxhlet extraction with ethanol/toluene (1:2, v/v) for 8h. The ash content, extractives, Klason lignin, holocellulose, cellulose, and pectin were determined following the TAPPI methods (T 211 om-93, T 204 cm-97, T 13, T 9, T 17). The cellulose content was determined by treating extractives-free samples with alcoholic nitric acid (4:1, v/v) solutions under reflux during four cycles of 1h. At the end, to calculate the final content of cellulose, one must subtract the ash content, because the banana pseudo-stem is rich in ash. The cellulose content was determined following the Kurschner-Hoffner approach, which consists of treating 1g of extractives-free samples with 25ml of alcoholic nitric acid solutions under reflux during four cycles of 1h. After each cycle, the solution was removed for a fresh volume. The alcoholic nitric acid solution involved mixing one volume of 65% (w/w) solution of nitric acid with four volumes of 96% purity ethanol. Hollocellulose was determined following the method of sodium chlorite, which consisted of 2g of extractives-free fractions with the mixture of 65ml distilled water, 0.5ml acetic acid, and 0.6g pure sodium chlorite during 1h at 75˚C. This treatment was repeated three times until the samples became white. The final content of holocellulose was calculated by subtracting the ash content due to the high ash content in the pseudo-stem.

The polysaccharides of the banana pseudo-stem materials were also analyzed. The holocellulose of banana pseudo-stem was mixed with 72% H_2SO_4 in 30˚C for 2h, and then subjected to a hydrolytic process with 4% H_2SO_4 in 120˚C for 1h. Lastly, the monosaccharide in the hydrolyzate was determined by Ion Chromatography (IC) (Dionex, USA).

Observation with LM

The samples of banana pseudo-stem were heated in boiling water to drive the internal air out, and then immersed in a mixture of 30% H_2O_2 and acetic acid at the ratio of 1:1(v/v). This process was carried out at 60˚C for 48h until the fibers became white and dispersed, and the fibers were carefully preserved for LM and CLSM observation. In the end, some fibers were removed from the suspension and placed with a dropper onto the glass slide, and then covered by a piece of coverglass and stained with Herzberg dye. At last, the sample of fibers from banana pseudo-stem was analyzed under the Olympus bx51 light microscopy (Olympus, Japan).

Observation with CLSM

Before they were fixed on the glass slide, the banana pseudo-stem fibers were preliminarily stained with acridine orange (AO). It was found that lignin contains a detectable level of fluorochromic activity that can be detected by selecting suitable incident and observed wavelength. It was reported (Li and Reeve 2004) that when the concentration of AO is sufficiently low, only lignin will be labeled and the fluorescence intensity of stained fibers can be correlated to the lignin distribution of fibers. Some wood materials have been studied with CLSM (Xu et al. 2006), but the lignin distribution in non-wood materials have never been reported. In this experiment, the banana pseudo-stem fibers were immersed in the solution consisting of 1mg AO powder and 10ml deionized water for 1h at room temperature. After fluorescent staining, the residual dye was washed out with deionized water, and the washing was conducted three times. Finally, the fluorescently labeled fibers were mounted on glass slide and observed under CLSM immediately to avoid fluorescence quenching.

Images were obtained using a Leica TCS SP5 CLSM device (Leica, Germany). An argon ion laser (488nm) was used as the illumination light.

Observation with SEM

Different morphological parts of banana pseudo-stem were removed and freeze-dried for SEM analysis. The samples of different parts were carefully cut to expose the inner wall and ektexine, and then the specimens were coated with a thin gold-palladium film. The observation was carried out under an LEO 1530VP SEM (Oxford, United Kingdom).

RESULTS AND DISCUSSION

Main chemical compositions, such as cellulose, holocellulose, lignin, extractives, and ash content of banana pseudo-stem were determined in the chemical analysis. In addition, the holocellulose compositions were also determined with IC.

Chemical Composition

The moisture content of the fresh banana pseudo-stem was about 96%. The amounts of chemical compositions in the raw material are listed in Table 1. In comparison with the traditional raw materials used in the pulp and papermaking industry, it was found that the content of holocellulose in banana pseudo-stem was much lower than wood fibers (Gong 2007; Cai and Tao 2007), but still higher than straw, which is a typical kind of nonwood fiber (Liu et al 2003). However, banana pseudo-stem had lower lignin content than wood and straw. While the ash and extractive contents in banana pseudo-stem were higher than that of wood fibers, they were still lower than straw. In conclusion, the banana pseudo-stem has potential in application of pulping and papermaking because of its acceptable content of cellulose and holocellulose and low lignin content.

The holocellulose composition of banana pseudo-stem is shown in Table 2. Glucose was the predominant monomer in this raw material with a 71.76% content, followed by xylose, 11.20%; arabinose, 7.34%; galactose, 2.02%; mannose, 0.58%; and galacturonic acid, 7.09%. Altogether, 97.90% of the holocellulose was composed by these monosaccharides.

Table 1. Chemical Composition* of Banana Stem and Some Other Raw Materials

(%)	Cellulose	Holocellulose	Klason lignin	Acid-soluble lignin	Ash content	Extracts	Pectin
Banana stem	39.12	72.71	8.88	1.90	8.20	3.05	0.27
Aspen (Gong,2007)	45	77.64-79.22	22.37-23.40	2.05-2.38	0.52-1.03	2.00-2.20	-
Straw (Liu et al. 2003)	36.20	63.1	11.30	4.15	12.87	7.45	-
Pine (Cai and Tao 2007)	45	71-83	24.57-29.85	0.37	0.27-0.28	1.11-3.51	-
* The composite contents are based on dried raw materials							

Table 2. Monosaccharides Composition of Banana Pseudo-Stem and holocellulose (qualitative proportion, %)

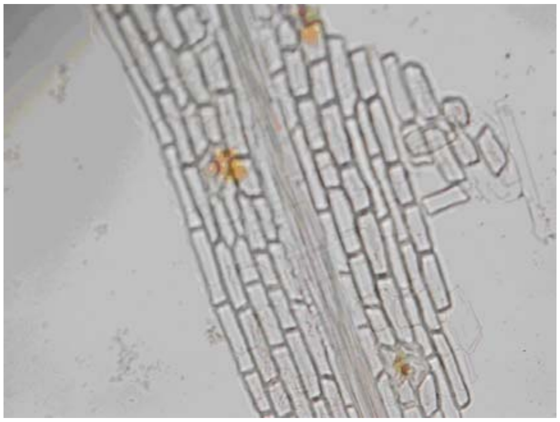
Sample	Glu	Xyl	Gal	Ara	Man	Glucuronic acid	Galacturonic acid
Pseudo-stem	71.76	11.20	2.02	7.34	0.58	n.d.	7.09
Holocellulose	67.90	12.58	2.25	5.44	1.02	1.27	5.44

LM Analysis

Figures 1 and 2 are LM micrographs of banana pseudo-stem. From Fig. 1 it is obvious that plenty of parenchyma cells were contained in the banana pseudo-stem, which caused high ash content and negative effects on the pulping and papermaking processes. It can be discovered from Fig. 2 that the pseudo-stem fiber is narrow and has a segmented structure (B in Fig. 2). The fiber has a regular shape, and the end (A in Fig. 2) is sharp, which agrees with the typical features of bast fibers (Wang 1999).

Observations with SEM and CLSM

It was reported by Ganan (2004) that the fiber bundles of banana pseudo-stem are covered with a layer of membrane composed of hemicellulose and pectin. After removal of these non-cellulose components, the fiber bundles present a complex hierarchically microordered structure formed by microsized fibers with two orientations. The fibers constructing this structure can be divided into elementary fibers and narrow fibers according to their orientation. The elementary fibers, in the diameter range of 10 to 15μm, are oriented in the bundle direction, while the narrow fibers in the diameter range of 3 to 4μm are intertwined around the elementary fibers.



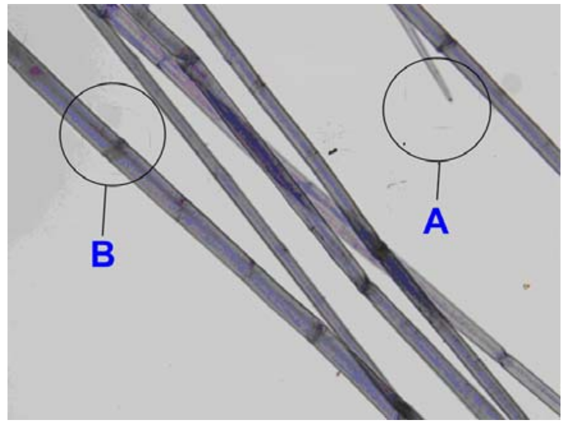
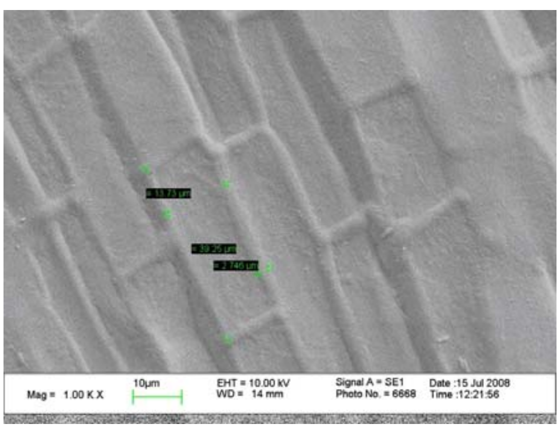


Fig. 1. Parenchyma cells (LM×200) **Fig. 2.** Pseudo-stem fibers (LM×200)

Even with the membrane covering the fibers, it is possible to determine the lignin distribution using CLSM, because the laser can be projected into a certain depth of the sample. The SEM and CLSM images (Fig. 3 and Fig. 4) of ektexine revealed a brick-like structure of fibers under the membrane, and this brick-like structure in Fig. 3 is 13.73µm in width, and the diameter of the frame is 2.746µm, which is the size a regular elementary fiber measured by LM. It can be concluded that this structure is comprised of both elementary fibers and narrow fibers.



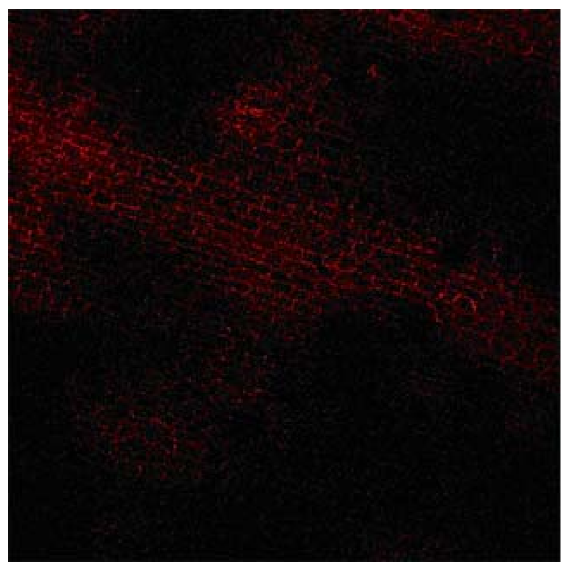


Fig. 3. Ektexine (SEM×500) **Fig. 4.** Lignin distribution (CLSM×100)

Figure 5 is a micrograph of the horizontal cross section of banana pseudo-stem. In this micrograph a film structure with plenty of holes can be observed. These films divide the pseudo-stem into innumerable elements for water retention. The diameters of the holes in the film vary from 20 to 40µm according to their location. This film structure, which has never been reported so far, is supposed to control the transportation and distribution of water in the whole banana pseudo-stem, which makes the banana plant tolerant of high temperatures.

The structure of banana pith, which is shown in Fig. 6, is significantly different from banana pseudo-stem. In the banana pith, there is no elementary fiber. The narrow fibers construct some pipes rather than fiber bundles. This structure, which has never been reported before, is considered to have a positive effect on the transportation of water in pith.

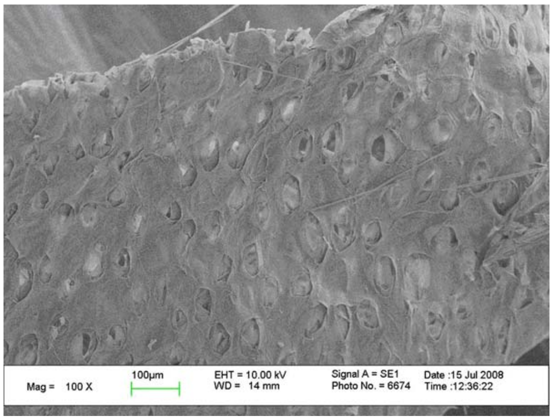


Fig. 5. Film structure (SEM×100)

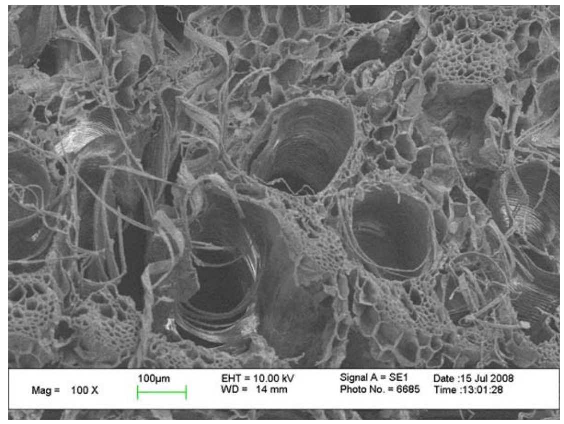


Fig. 6. Banana pith (SEM×100)

The fibers with diameters in the range 3 to 5μm are much narrower than the elementary fibers mentioned above, and the narrow fibers are shown in Fig. 7. In the image captured by CLSM (Fig. 8), both the elementary fibers and the narrow fibers can be clearly observed. The diameter of the elementary fiber is 21μm, while the diameter of a narrow fiber is just 6μm. These results are compatible with the observations made by Ganan (2004).

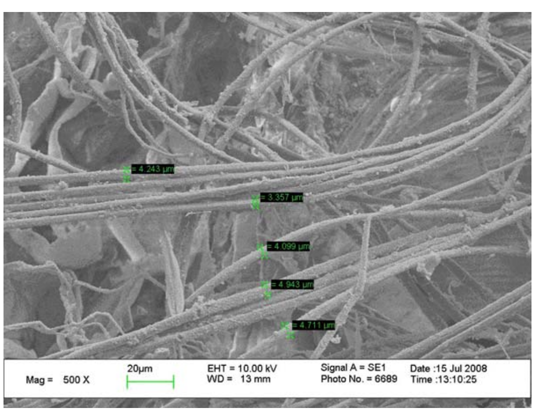


Fig. 7. Narrow fiber in pith（SEM×500）

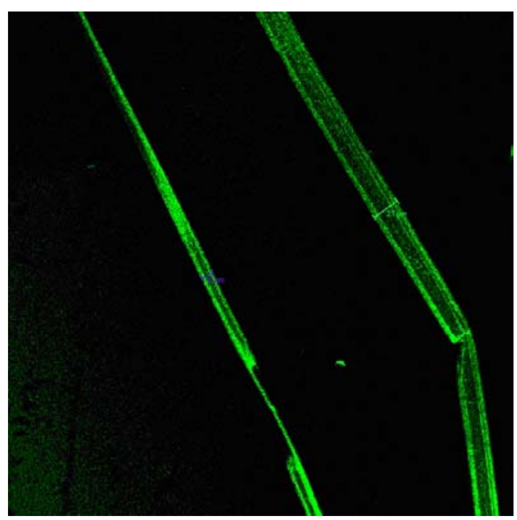


Fig. 8. Pseudo-stem fibers (CLSM×400)

CONCLUSIONS

1. Banana pseudo-stem has a high holocellulose content and low lignin content, thus making it an ideal material for application in pulping and papermaking. The monomeric content of holocellulose of banana pseudo-stem consists mainly of glucose (71.76%), followed by xylose, 11.20%; arabinose, 7.34%; galactose, 2.02%; mannose, 0.58%; and galacturonic acid, 7.09%.
2. Banana pseudo-stem has unique morphological features. Fiber bundles in banana pseudo-stem are covered by a non-cellulose membrane, and are constructed by two kinds of fibers: elementary fibers with diameters of 10~15μm and narrow fibers with diameters of 3~4μm.
3. There is no elementary fiber in banana pith. The narrow fibers resemble pipes rather than fiber bundles. This structure, which has never been reported before, is considered to facilitate the transportation of water in the pith.

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