Nanoscale Characterization of Reed Stalk Fiber Cell Walls

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Reed (*Phragmites australis*) is a natural biological material that has great potential as reinforcing material in bio-composites. In order to evaluate the potential of reed stalk for reinforcement, the microstructure, elemental composition, microfibril angle (MFA), and mechanical properties of fiber cell walls were investigated by means of scanning probe microscopy (SPM), energy dispersive analysis of X-rays (EDAX), X-ray diffraction (XRD), and nanoindentation, respectively. The effects of elemental composition and microfibril angle of reed fibers on the mechanical properties were also considered. The results indicated that reed fiber cells have a multilayered structure. The observed increase in lignin content and decrease in MFA may contribute to the increase of mechanical properties. The elastic modulus and hardness of fibers in the upper part of the reed stalk were higher than those of the lower part. Based on nanoindentation results found in the literature, reed fibers have higher elastic modulus and hardness than poplar and spruce fibers.

Keywords: Reed stalk; Fiber cell wall; Elemental composition; Microfibril angle; Mechanical properties; Nanoindentation

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INTRODUCTION

With the rapid development of wood-based composites in recent years, more and more wood processing enterprises are facing raw material shortages due to the global decline in forest resources. Bio-fibers from agricultural residues are an abundant, inexpensive, widely distributed, recyclable, versatile, biodegradable, and readily available source of renewable lignocellulosic biomass (McKendry 2002; Liu *et al.* 2005). Therefore, increasing attention has been paid to non-wood, lignocellulosic biomass, such as wheat straw, cotton stalks, and waste grass clippings, for use in composite manufacturing (Nemli *et al.* 2009).

Reed (*Phragmites australis*) is one of the most widely distributed wetland plant species on Earth. Due to its fast-growing properties and high biomass yields, reed is recognized as a promising source of renewable energy (Lewandowski *et al.* 2003). Reed has traditionally been used to make paper (Shatalov and Pereira 2006; Abrantes *et al.* 2007), but recently other uses are being considered. Being a typical natural biological material, reed has the potential to be used as raw material in the wood-based composites industry (Nourbakhsh and Ashori 2010; Garcia-Ortuno *et al.* 2011). To utilize reed efficiently in composite products, it is necessary to better understand the strength properties of the individual fibers at the microscopic level. However, the small size of the structures makes testing individual cell compounds difficult with conventional measurements.

The nanoindentation technique is a method that can be used to measure the mechanical properties of a variety of materials at the micron or submicron scale without any extraction or damage to the material. It was introduced in the study of wood cell wall mechanics by Wimmer *et al.* (1997). Since then, considerable work has been carried out in order to develop the technique and to determine the mechanical properties of various bio-fibers or wood cell walls. Gindl and Schöberl (2004) measured the mechanical properties of spruce cell walls using nanoindentation. The results indicated that lignification of spruce tracheid secondary cell walls was related to longitudinal hardness and modulus of elasticity. Tze *et al.* (2007) have worked on wood cell walls and they have shown a small length-scale effect during loading and a decrease of elastic modulus and hardness with an increase of the microfibrillar angle (MFA). Wu *et al.* (2010) evaluated the elastic modulus and hardness of crop stalk cell walls using nanoindentation. They found that crop stalk fibers have better mechanical properties than wood. However, there are no reported data on using this technique to evaluate the mechanical properties of the fiber cell walls of reed.

In the present study, the microstructure of the fiber cell wall of reed stalk was characterized by scanning probe microscopy (SPM). The elemental composition, MFA and mechanical properties of fiber cell wall were determined using energy dispersive analysis of X-rays (EDAX), X-ray diffraction and nanoindentation, respectively. Their relationships were also investigated.

EXPERIMENTAL

Materials

Reed (shown in Fig. 1a) used in the study was collected from Jiangsu Province, China. It is a tall and upright perennial grass with a stem height of 1 to 3 m and a diameter of 8 to 20 mm. The reed stalk was dried in ambient condition to about 10% moisture content. Previous research has shown that the chemical, physical, and mechanical properties vary significantly between the bottom and top sections of biomass stalks (Li *et al.* 2013). Thus, the samples for analysis were selected from both the lower and upper parts of dried reed stalk. Two sampling areas were defined on one stem, and the locations are shown in Fig. 1b.

Methods

Elemental analysis of reed fibers by SEM-EDAX

As seen in Fig. 1c, samples were cut to dimensions of 2 × 5 mm (width by length) with the natural thickness of reed stalk, using sharpened blades. Each sample was then sputtered (in the cross-section) with gold-platinum coating using a sputter coater. Elemental analysis of the mature fibers was performed by a scanning electron microscope (SEM, FEI Quanta200) equipped with energy dispersive analysis of X-rays (EDAX, Genesis XM2). Ten samples on each part of the stalk were examined.

Measuring microfibril angle of reed fibers

The tilt angle of the cellulose fibrils with respect to the longitudinal cell axis, often called the microfibril angle (MFA), can vary considerably within a single individual plant. To investigate the relationship between the microfibril angle and the mechanical properties, the MFA of mature fibers from the upper and lower parts of reed stalk were

determined using X-ray diffraction (XRD). Samples were cut to dimensions of 3 × 10 mm (width by length) with a thickness of 0.5 mm, using sharpened blades. XRD measurements were obtained using a Rigaku Ultima IV difractometer, equipped with a Cu Kα radiation source, operating at 40 kV and 30 mA. The XRD pattern was recorded within an angle range from 90° to 270°, at a step size of 0.36°. The mean microfibril angle was determined according to a method developed by Cave (1966) and Meylan (1967). Ten samples on each part of the stalk were examined.

Nanoindentation testing

The samples for nanoindentation were also selected from the lower and upper parts of reed stalk. They were cut into small blocks with dimensions of 2 × 5 mm (width by length) with the natural thickness of the stalk. The small blocks were embedded in epoxy resin, which was formulated of cycloaliphatic epoxide resin (ERL-4221) (2.5 parts), polycoldieposide (DER-736) (1.5 parts), nonenyl succinic anhydride (NSA) (6.5 parts), and dimethylaminoethanol (DMAE) (0.1 parts). The embedded blocks were placed in a desiccator under vacuum for 12 h, and were then cured in an oven at 70 °C for 8 h. This sealing procedure made it easier to mount the reed stalk inside the embedding mold parallel to the longitudinal axis of reed cell wall. The cured specimens were then mounted onto an ultramicrotome equipped with a diamond knife. Finally, a smooth surface of the specimen was obtained by diamond knife cutting.

The nanoindentation test was performed with a Triboindentor, in conjunction with SPM (Hysitron Inc., USA) at an ambient temperature of 20 °C and relative humidity of 25 ± 4%. A Berkovich indenter, a three-sided pyramid with an area-to-depth function, was used for all experiments. All of the samples were put into the Triboindentor chamber for at least 24 h before indentations were performed. The indentation experiment included four parts. First, the approach was performed with an indenter surface approach rate of 10 nm/s. Second, once the tip contacted the sample surface, a constant displacement rate of 5 nm/s was applied until the target indentation peak load of 150 μN was reached. Third, at this peak load, the loading was held for 5 seconds to avoid the effect of creep occurring in viscous material during the unloading (Liu *et al.* 2006). Finally, the unloading was executed at the same loading rate, namely 5 nm/s. At the end of the experiment, the specimens were examined by SPM, which is capable of evaluating the position and quality of the indentations. Thirty to forty indentations were made on a cross-section of six to ten mature cell walls for each sample (shown in Fig. 1d and e).

The elastic modulus and hardness were deduced from the relationship to contact stiffness and the contact area between indenter and surface, as discussed in detail by Oliver and Pharr (1992). The hardness can be obtained from Equation 1,

$$\mathrm{H} = \frac{P_{\max}}{A} \quad (1)$$

where H is hardness, P_{max} is the peak load, and A is the projected contact area at peak load.

As the indenter was allowed to penetrate into the sample, both elastic and plastic deformation occurred, and only the elastic portion of the displacement was recovered during unloading. The elastic modulus of the specimen was inferred from the initial unloading contact stiffness (S). The relationship among contact stiffness, contact area, and elastic modulus was derived as follows:

$$S = 2\beta E_r \sqrt{\frac{A}{\pi}} \tag{2}$$

where β is a constant that depends on the geometry of the indenter (β = 1.034 for a Berkovich indenter) and E_r is the reduced elastic modulus that accounts for the fact that elastic deformation occurs in both the tested specimen and the indenter.

The specimen's Young's modulus (E_s) was then calculated by Equation 3,

$$E_s = \left(1 - \nu_s\right)\left(\frac{1}{E_r} - \frac{1-\nu_i}{E_i}\right)^{-1} \tag{3}$$

where E_s and ν_s are the elastic modulus and Poisson's ratio for the specimen, and E_i and ν_i are the same quantities for the indenter, respectively. For the diamond indenter, E_i equals 1141 GPa and ν_i equals 0.07. In order to calculate a sample's elastic modulus, the Poisson's ratio of reed stalk was assumed to be 0.25, to achieve a consensus with Wu *et al.*'s results (2009).

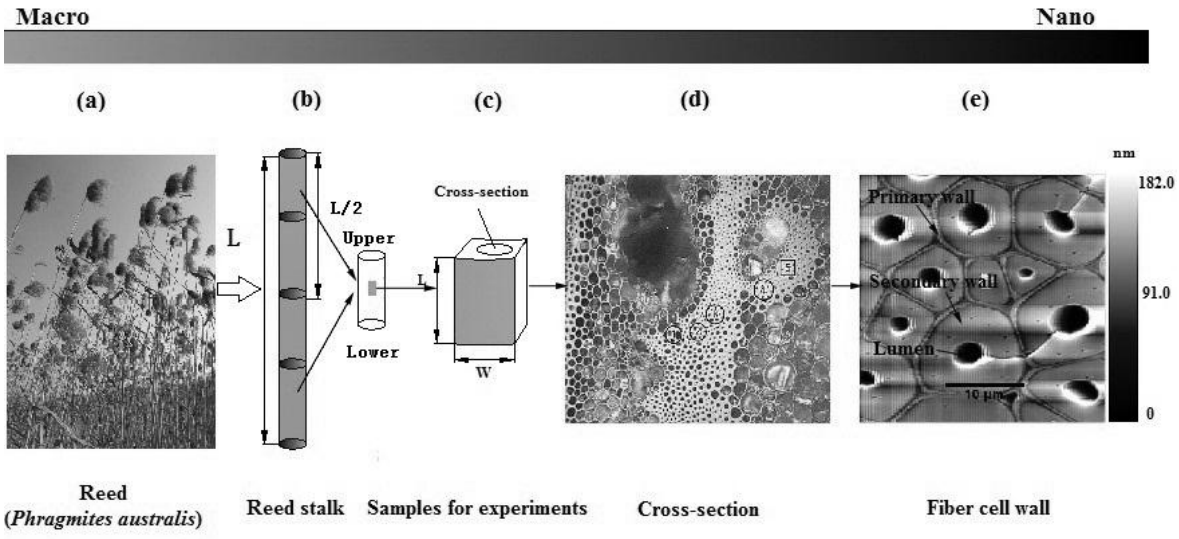

Fig. 1. Measuring of reed stalk fiber: (a) the reed plant, (b) two groups of samples of reed stalk, (c) small samples for experiments, (d) the cross structure of reed stalk, (e) the microstructure of reed fiber cells

RESULTS AND DISCUSSION

Microstructure of Reed Fibers

Figure 1 shows the hierarchical organization of reed fibers in different size scales. Figure 1d presents the cross-section images of samples. The marked regions were selected for nanoindentation. Figure 1e is the SPM image of reed fiber cells with a scanning size of 40 × 40 μm. Image processing software, equipped in scanning probe microscopy, was used for evaluation of the SPM images. After detection by the software, each individual aggregate area was calculated in pixels, which then was transformed to

μm. Through evaluation by the software, it was discovered that the reed fiber is an irregular polygon with a diameter of 6 to 33 μm. The mean diameter of the fiber was 13.4 μm. Individual fibers exhibited a multilayered structure, consisting of several layers of cell wall and a lumen at the center. The fiber cell wall also exhibited a multilayered structure with alternating broad and narrow lamellae. The primary wall in the outer layer of cell wall was thin, with a thickness of about 0.7 μm. The secondary wall also exhibited a multilayered structure and made up approximately 80% of the cell wall volume, exerting a dominant influence on the mechanical properties of the composite cell wall, due to its stiffness. Therefore, the nanoindenter tip was positioned on the secondary cell wall of reed fibers to perform nanoindentation tests.

Major Elements of Reed Fibers

As seen in Fig. 2a, the regions marked on the fibrous tissue of reed stalk were randomly selected for scanning. A representative EDAX spectrum is given in Fig. 2b, and the spectrum clearly indicated the presence of carbon and oxygen in reed fibers, because C and O photoelectron peaks were clearly resolved. Only these two elements were present in a noticeable amount. The high content of C and O can be explained by the fact that reed is a cellular biomaterial with an intricate structure, principally consisting of three biopolymers: cellulose, hemicelluloses, and lignin (Nourbakhsh and Ashori 2010). A very small peak due to Si was also present in the survey spectra. It is reported that the silicon is mainly absorbed by reed from the vegetative wetland soil (Struyf *et al.* 2007).

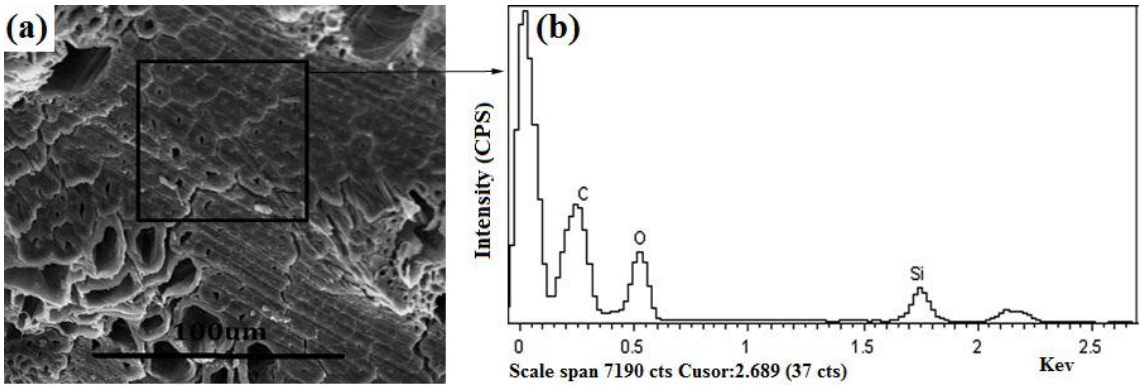

Fig. 2. SEM-EDAX analysis of reed fibers: (a) regions selected for elemental analysis in reed stalk, (b) a representative EDAX spectrum of reed fibers

Elemental weight percentage and atomic percentage values of reed fibers are presented in Table 1. It can be found that the major elements are C, O, and Si and that the contents of these elements vary between the upper stalk and lower stalk. The oxygen-to-carbon atomic ratio (O/C) was 0.95 for the lower stalk (calculated from Table 1) and was higher than that for the upper stalk, which meant that the lignin or extractives content of fibers in the upper stalk was higher. An increasing O/C atomic ratio correlates to a decreasing lignin or extractives content (Gustafsson *et al.* 2003). In order to confirm the results of the elemental analysis, the lignin content of reed stalk was tested with a chemical method (GB/T 2667.8-1994). Results showed that the average lignin content of the upper stalk was 184 $g \cdot kg^{-1}$, which was higher than the measurement of the lower part, 161 $g \cdot kg^{-1}$.

In addition, the weight percentage of Si of fibers in the lower stalk was 0.92%, which was higher than 0.55% for the upper stalk. In previous research, the element

silicon was contained in reed stalk as the compound silica (SiO_2) (Link *et al.* 2010). The silica contents of fibers in the upper and lower stalks were 1.18% and 1.97%, respectively, evaluated according to the weight percentage of silicon (Si). This phenomenon can be explained by the fact that silicon can partly participate in the mineral cycle in reed stalk through diffusion and transpiration. A higher content of silicon in the lower stalk can prevent water from permeating into the stalk to keep it growing (Pan 2004).

Table 1. Elemental Analysis of Reed Fibers

Elements	Upper stalk		Lower stalk	
	Weight percentage	Atomic percentage	Weight percentage	Atomic percentage
C	45.66 (0.45)	53.09 (0.42)	44.03 (0.40)	51.34 (0.40)
O	53.37 (0.33)	46.47 (0.24)	55.01 (0.40)	49.01 (0.40)
Si	0.55 (0.01)	0.27 (0.01)	0.92 (0.08)	0.46 (0.04)
The values in parentheses are standard deviations				

Microfibril Angle of Reed Fibers

The microstructure of reed fiber showed that the secondary wall is the thickest layer of all the cell wall layers. Thus, the microfibril angle (MFA) in this layer was measured in this experiment. The MFA is influenced by various factors, including the plant variety, the plant part, the growing environment of the plant, and others (Lasserre *et al.* 2009; Lindstrom *et al.* 1998). The 002 peak intensities in the diffraction patterns were fit into Gaussian curves using a nonlinear least-squares algorithm with the software program Microcal ORIGIN to obtain the MFA. X-ray diffraction diagrams of reed fiber cell walls are given in Fig. 3, and the spectrums clearly showed the small variation of MFA along the height direction of reed stalk. The mean MFA of the upper stalk was 11.1 ± 0.5°, which was smaller than the measurement of the lower part, 11.6 ± 0.4°.

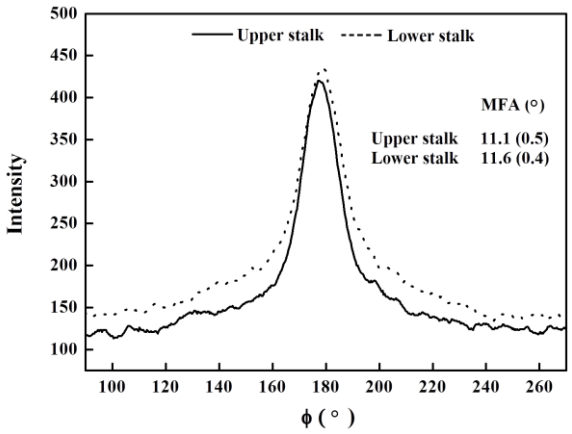

Fig. 3. X-ray diffraction diagrams of reed fiber cell walls

Mechanical Properties

Figure 4 shows a representative indentation load-displacement curve of the fiber cell wall. The nanoindentation contact depth reached the peak displacement of 100 nm before unloading. Once loading force was removed, a displacement of about 72 nm still remained, which accounted for 72% of the total depth, meaning the elastic recovery rate was only 28%. The reason for this phenomenon is related to the plastic deformation that occurred when the indenter tip was inserted in the microfibrils of the secondary cell wall

layer. The plastic deformation inhibited the elastic recovery of lignin-hemicellulose complexes.

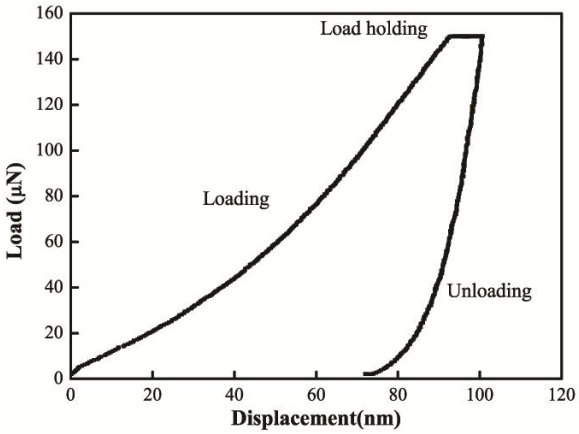

Fig. 4. A representative nanoindentation load-displacement curve of reed fiber cell walls

The values of elastic modulus and hardness of fiber cell walls in reed stalk are shown in Table 2. The upper stalk presented higher mechanical property values than the lower part. Elastic modulus and hardness of the fiber cell wall in the upper stalk were 20.97 GPa and 0.53 GPa, which were 12.3% and 15.2% higher than those of the lower stalk, respectively.

The mechanical properties of fiber cell wall are affected by a number of factors, such as the density, the MFA, the chemical composition, the kind of plant, and others (Li *et al.* 2013). The MFA is considered to be the predominant factor for the mechanical properties of single fibers (Page *et al.* 1977; Tze *et al.* 2007); in other words, as the MFA of cell wall increased, the mechanical properties decreased. The results of the present study agreed with the findings that the hardness and elastic modulus decreased with increasing MFA, as reported by Tze *et al.* (2007).

The chemical composition can also affect the mechanical properties of cell walls. Gindl *et al*. (2002) suggested that lignification has an obvious effect on the mechanical properties of cell wall. During lignification, the spaces between cellulose-hemicellulose strands and possibly part of the hemicellulose sheath covering the cellulose are filled with lignin. Filling the existing spaces with lignin certainly increases the overall stiffness of the structure. As mentioned before, the lignin content of reed fibers in upper stalk was higher than that of the lower part. This agreed with the results of Gindl *et al.* (2002), who observed that increasing lignin content might contribute to a higher measured hardness. In addition to the lignin, the research work of Schmidt *et al.* (1999) and Wu *et al.* (2010) indicated that higher silica (SiO_2) content results in higher stiffness and lower flexibility. In this study, the lower part of reed stalk with a higher content of silica presented a lower mechanical properties. This could be attributed to the fact that the silica content of reed stalk was far less than lignin. So in this case silica did not play an important role with respect to mechanical properties of reed stalk fiber cell wall, compared to lignin.

Furthermore, the high humidity growing environment may be another influential factor, contributing to the fact that the lower stalk has lower mechanical property values than the upper part. Similarly, the mechanical properties of fibers' cell walls in the upper silvergrass stalk are better than those of the lower stalk (Liao *et al.* 2012).

Table 2. Mechanical Properties of Fiber Cell Walls of Reed Stalk

Materials	Elastic modulus (GPa)	Hardness (GPa)
Reed stalk (upper)	20.97 (1.37)	0.53 (0.02)
Reed stalk (lower)	18.67 (0.83)	0.46 (0.04)
Mean	19.82	0.50
The values in the parentheses are standard deviations		

Figure 5 lists the previous research on the mechanical properties of poplar (Wu *et al.* 2009), spruce (Gindl and Schöberl 2004), and rice straw (Wu *et al.* 2010), as measured by nanoindentation. Compared with the reference data, the mean value of the elastic modulus of reed fiber cell walls (19.82 GPa) was comparable to that of rice straw (19.40 GPa) but higher than that of poplar (16.90 GPa) and spruce (16.50 GPa). It can also be seen that the highest mean value of hardness (0.50 GPa) was observed in the cases of reed and rice straw, followed by 0.49 GPa in poplar, and 0.34 GPa in spruce.

In general, there are many factors affecting the properties of fiber cell walls, especially among different plant species (Wu *et al.* 2009). The MFA and chemical composition were just the part reasons related to the differences in the mechanical properties of cell walls among reed, poplar and spruce. The mean MFA of reed fiber can be up to approximately 11.4°, which is much lower than those of poplar (Fang *et al.* 2006) and of spruce (Peura *et al.* 2008). Besides, reed stalk usually has higher silica content than the two species of wood (Cornelis *et al.* 2010). Therefore, when compared with poplar and spruce, the mechanical properties of reed stalk fiber cell walls were higher. This agreed the results in the experiments referenced. Altogether, reed is a natural source of cellulosic fiber and has great potential as reinforced material in bio-composites.

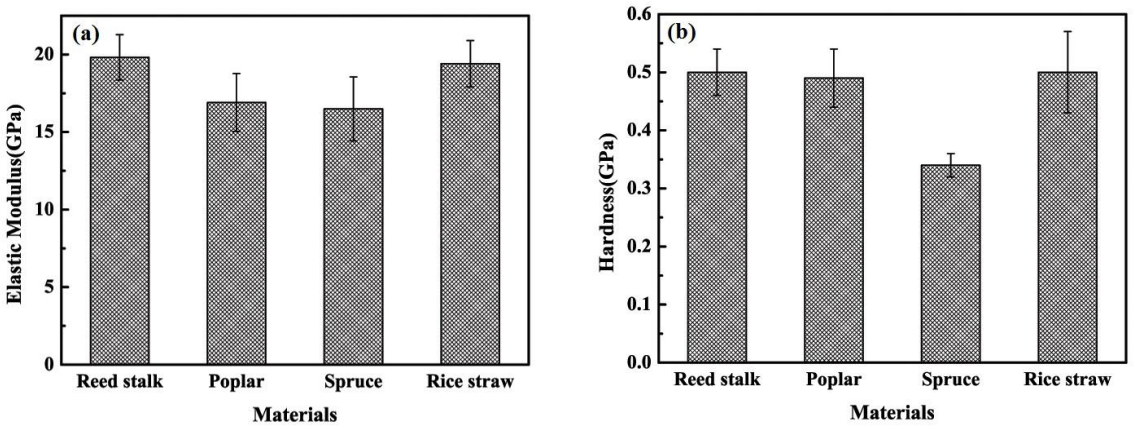

Fig. 5. Results of nanoindentation testing: (a) comparison of mean value of elastic modulus in reed stalk and other materials, (b) comparison of mean value of hardness in reed stalk and other materials

CONCLUSIONS

Within the microstructure, the elemental composition, microfibril angle (MFA), and mechanical properties of fiber cell walls were investigated and the following conclusions can be drawn:

1. Reed is a highly organized, multilayer composite. The fiber cell of reed stalk is an irregular polygonal with a diameter of 6 to 33 μm.
2. Based on the results of SEM-EDAX analysis, the major constitutive elements of reed

fibers were C, O, and Si. The degree of lignification of fibers in upper stalk was higher than the lower stalk. In contrast, the content of SiO_2 (silicon compound) of fibers in upper stalk was lower.

3. There is a small variation of microfibril angles in one stem of reed stalk. The mean MFA of fibers in the upper and lower parts of reed stalk were 11.1° and 11.6°, respectively.
4. At the cell wall level, the elastic modulus and hardness obtained by nanoindentation were more related to the properties of natural fibers. Higher lignin content and smaller MFA may contribute to the higher mechanical properties of reed fibers. Compared to lignin, silica has no an obvious influence on the mechanical properties of fibers. Hardness values of the fiber cell wall in lower and upper stalks were measured to be 0.46 and 0.53 GPa, and elastic modulus values were 18.67 and 20.97 GPa, respectively. Moreover, reed fibers have better mechanical properties than poplar and spruce fibers.

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