

## Effects of Extraction Methods on Anti-Mould Property of Bamboo Strips

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To achieve improvements in anti-mould properties, bamboo strips were subjected to extractions with different combinations of extraction media: boiling water, 1.0 wt.% hydrochloric acid (HCl), or 1.0 wt.% sodium hydroxide (NaOH). The impacts of different extraction methods on the structure and anti-mould properties of bamboo strips were investigated. The results suggested that bamboo strips treated alternatively with different extraction media were free of changes in crystalline structure and cell morphology but showed partial degradations of hemicellulose and decreases in the amount of free hydroxyl groups. As a result of these treatments, elimination or migration of extractives (including starch, soluble saccharides, and aliphatics) improved the resistance of bamboo strips to *Penicillium citrinum*, *Trichoderma viride*, *Aspergillus niger*, and a mixture of these mildews. Among the tested treatments, sequential extraction with boiling water, 1.0 wt.% NaOH, and 1.0 wt.% HCl resulted in a mildew-preventing efficiency of 96.9%, indicating optimum anti-mould properties.

*Keywords:* Bamboo strips; Extraction; Medium; Extractive; Anti-mould properties; Impacts

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### INTRODUCTION

China ranks first in the world in area of bamboo forests and bamboo yield and occupies a leading position in bamboo processing and utilization (Du *et al.* 2016; Li *et al.* 2016; Yu *et al.* 2016; Zhou *et al.* 2016). The inclusion content of bamboo is greater than that of wood by a factor of 1.5 to 2, and the total amount of starch and water-soluble saccharides is as high as 4 to 6% (Wang *et al.* 2000). The significant positive correlation between the amount of starch/water-soluble saccharides and mildew growth renders bamboo more vulnerable to mildew than wood (Min *et al.* 1997). Mildew causes surface contamination of bamboo, which can reach several millimeters in depth in severe cases and is very difficult to remove. Consequently, mildew greatly damages the appeal and compromises the value of bamboo materials (Wang *et al.* 2000). According to incomplete statistics, 10% of China's annual bamboo yield is lost due to decay and mildew (Zhao *et al.* 2010). Consequently, fungus resistance treatments are critical for the efficient utilization of bamboo resources. There have been many investigations of fungus resistance treatments (Sun and Duan 2004; Zhao *et al.* 2010; Li *et al.* 2015; Yu *et al.* 2016). An effective approach to prevent bamboo mildew is to eliminate the required conditions for mildew growth, including the nutrients, temperature, and optimal humidity. Extraction treatments remove large amounts of nutrients including starch, saccharides, proteins, and aliphatics from bamboo and provide effective resistance against mildew. Bamboo has been extracted with cold water, hot water, sodium

hydroxide (NaOH) solutions, benzene, and alcohols, which all improve the anti-mould properties (Sun *et al.* 2006; Guan *et al.* 2011; Zhou *et al.* 2012; Cheng *et al.* 2013; Fei *et al.* 2013; Guan *et al.* 2013; Ma *et al.* 2013; Huang *et al.* 2014; Jiang *et al.* 2015). However, most of these treatments were conducted using only a single extraction medium and could not completely remove the nutrients required for mildew growth. Thus, they resulted in limited mould resistance.

In the present study, bamboo materials were treated sequentially with different combinations of boiling water, NaOH, and hydrochloric acid (HCl), with the aim to remove as many nutrients as possible to improve their anti-mould properties. The impacts of these treatments on bamboo structure and anti-mould properties were investigated. Additionally, this work provides references for the development of effective anti-mould treatment procedures for bamboo materials.

## EXPERIMENTAL

### Materials

Natural color bamboo strips were purchased from Zhejiang Yongyu Bamboo Industry (Huzhou, China) and cut into specimens of 50 mm × 20 mm × 5 mm (length × width × thickness). Specimens were free of joints, with approximately 10% moisture content.

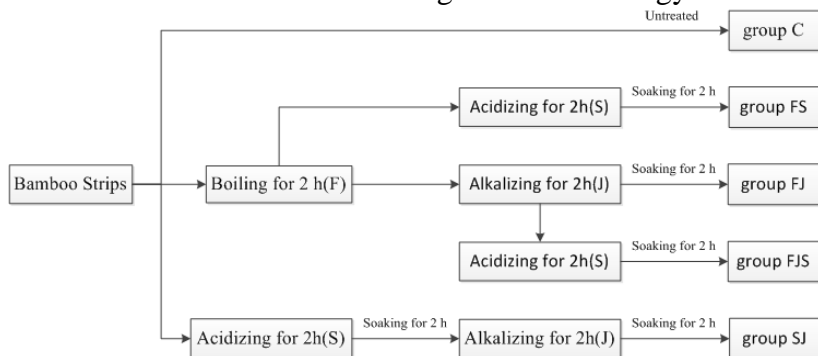
*Penicillium citrinum*, *Trichoderma viride*, and *Aspergillus niger* mildews were obtained from the microbiology laboratory of Zhejiang Agriculture and Forestry University.

For extraction treatment methods, 1.0 wt.% HCl and 1.0 wt.% NaOH solutions were used as extractive solvent. Deionized water was prepared in the lab.

### Methods

#### *Extraction of bamboo strips*

Bamboo strips were sequentially extracted with boiling water and 1.0 wt.% NaOH (group FJ), boiling water and 1.0 wt.% HCl (group FS), 1.0 wt.% HCl, soaked in deionized water for 2h, and 1.0 wt.% NaOH (group SJ), or boiling water, 1.0 wt.% NaOH, and 1.0 wt.% HCl (group FJS). These samples were compared with control groups without extraction (group C) to investigate the impacts of different treatments on the anti-mould properties of bamboo. The specimens were submerged into determined extractive solvent for 2 h. The reported results were averaged from 12 independent determinations. After treatment, the bamboo strips were soaked in deionized water for 2 h and then air-dried. The circuit diagram of technology is shown in Fig. 1.



**Fig. 1.** Technology roadmap of bamboo strips extraction

### *Fourier transform infrared spectroscopy (FT-IR) analysis*

Untreated natural bamboo or extracted with different medium combinations were ground to powder using a micro plant grinding machine (Tester Instruments Co., Ltd., Tianjin, China) and passed through a 300-mesh screen. The powder was oven-dried at 103 °C, mixed with potassium bromide at a 1:100 mass ratio, and pressed into even and thin chips. Molecular structures of bamboo strips were scanned on a Nicolet 6700 Fourier transform infrared spectrometer (Thermo Fisher Scientific, Shanghai, China) at 4 cm<sup>-1</sup> resolution in a wavelength range from 3750 cm<sup>-1</sup> to 750 cm<sup>-1</sup>. Each sample was scanned 32 times.

### *X-ray diffraction (XRD) analysis*

The phase composition of bamboo strips that were not treated or extracted with different procedures was analyzed using a XRD 6000 X-ray powder diffractometer (Shimadzu, Kyoto, Japan). The X-ray tube was equipped with a copper target and set to a voltage of 40 kV and electric current of 30 mA. Samples were scanned with 2 $\theta$ / $\theta$  linked-scanning at a speed of 2 °/min in the scanning area of 5° to 40° (2 $\theta$ ).

### *Microstructure of bamboo strips*

Untreated natural bamboo or extracted with different procedures were cut into slices and the transverse sections were coated with gold for 60 s. The microstructures of bamboo strips were observed under a MT-1000 scanning electron microscope (SEM; (Shimadzu, Kyoto, Japan)) with an accelerating voltage of 5.0 kV.

### *Anti-mould properties of bamboo strips*

To prepare the culture plates, 200 g of peeled and washed potato was cut into slices and boiled in 1000 mL water for about 40 min. After cooling to room temperature, the boiled potato was filtered. The filtrate was brought to 1000 mL with water and mixed with 20 g of glucose and 23 g of agar powder. The mixture was boiled and then aliquoted to three 500 mL Erlenmeyer flasks. The flasks were sealed with plugs and water-proof paper and autoclaved at 0.1 MPa and 121 °C for 30 min. The autoclaved culture medium was transferred into a tissue culture hood and poured into sterilized 10 cm culture dishes (15 to 20 mL per dish). The culture plates were cooled and stored for later use.

Anti-mould tests were conducted on untreated and treated bamboo strips according to the GB/T 18261 (2013) standard. In-house tests were performed at 28 ± 2 °C and 85 ± 5% relative humidity. The growth of *Penicillium citrinum*, *Trichoderma viride*, *Aspergillus niger*, and mixed mildew (1:1:1 mixture of these three) on bamboo strips was recorded daily (Kositchaiyong *et al.* 2013). Pictures of the bamboo strips were taken at the end of the 28-day time course and analyzed to calculate the anti-mould properties.

One metric used for analysis of anti-mould properties was the infection ratio, *i.e.*, the ratio of the mildewed area out of the sample's total surface area. The infection ratio was assessed *via* observation.

The second measurement used was the anti-mould efficiency of extracted bamboo strips, calculated by Eq. 1 (GB/T 18261 (2013) standard),

$$E = \left(1 - \frac{D_1}{D_0}\right) \times 100\% \quad (1)$$

where  $E$  is the anti-mould efficiency (%),  $D_1$  is the average infection ratio of extracted specimens, and  $D_0$  is the average infection ratio of control specimens. The anti-mould efficiency of a specific group of specimens is defined as the mean value of their  $E$  values

against the three individual mildews and the mildew mix.

## RESULTS AND DISCUSSION

### FT-IR Analysis

The FT-IR spectra of treated specimens and the control group are shown in Fig. 2. Compared with untreated strips (C), the strips of groups FJ, FS, SJ, and FJS presented a decreasing signal at  $3435\text{ cm}^{-1}$ , caused by stretching vibration absorption of O-H bonds (Liu *et al.* 2015). The most likely reason for this decrease was that extraction caused breakage and reformation of hydrogen bonds in the specimens and reduced the amount of free hydroxyl groups. Decreasing the amount of free hydroxyl groups lowered the water-absorbing capability of bamboo strips, which contributed to improved anti-mould properties. In addition, after extraction, the samples FJ, FS, SJ, and FJS presented a notable decreasing trend of absorption at  $1740\text{ cm}^{-1}$  (Fang *et al.* 2015; Liu *et al.* 2015), and the absorption of SJ and FJS almost disappeared completely. Absorption at this wavelength originated from the stretching of fatty acids and acetyl and carboxyl groups in hemicellulose. Therefore, the observed changes suggested that after extraction, most of the polysaccharide in hemicellulose degraded and markedly decreased the water-absorbing capability of the sample, reducing the moisture available for mildew growth. At the same time, aliphatic and soluble saccharides in the bamboo strips were damaged or removed, thus eliminating the nutrients required for mildew growth. The FT-IR results suggested that extraction treatments improved the anti-mould properties of bamboo strips and that the FJS extraction procedure greatly increased the anti-mould properties of bamboo strips.

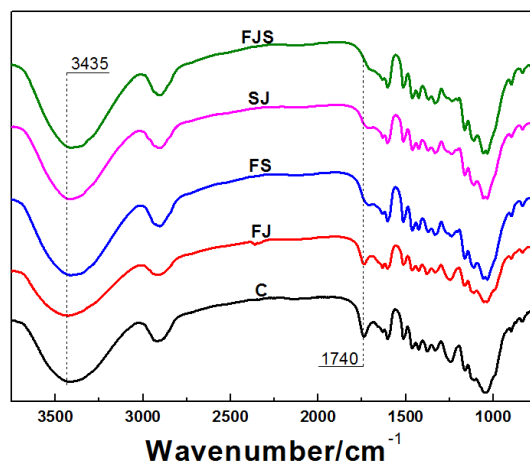


Fig. 2. FT-IR spectra of bamboo strips extracted via different procedures

### XRD Analysis

The XRD plots of the bamboo strips in groups FJ, FS, SJ, FJS, and control group C are shown in Fig. 3. The untreated and treated strips all presented diffraction peaks of the 101, 002, and 040 diffraction planes, which are the characteristic peaks of the cellulose crystals (Liao *et al.* 2002; Li *et al.* 2015; Chu *et al.* 2017). The presence of these peaks suggested that extraction did not alter the crystal structure of bamboo. Additionally, untreated and treated strips presented similar shapes of diffraction peaks of

the 101 and 040 diffraction planes, while diffraction peaks of the 002 diffraction planes were increasingly enlarged and sharpened for the FJ, FS, SJ, and FJS samples, suggesting an increasing trend of crystallinity in those extracted groups (Fang *et al.* 2015; Liu *et al.* 2015; Chu *et al.* 2017). The most likely reason for this trend was that extraction caused breakage of hydrogen bonds and/or re-orientation of molecules chains in cellulose, resulting in the formation of new crystalline regions and an increased crystallinity of cellulose. Consequently, the number of free hydroxyl groups in bamboo strips decreased, which greatly contributed to their anti-mould properties.

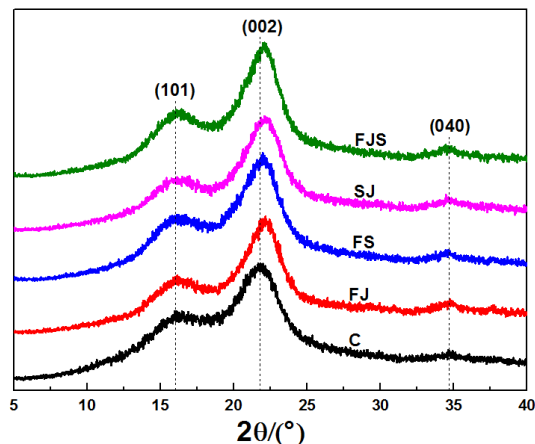


Fig. 3. XRD spectra of bamboo strips extracted via different procedures

### SEM Analysis

According to the analysis presented above, the FJS extraction procedure (boiling water, 1.0 wt.% NaOH solution, and 1.0 wt.% HCl) was able to remove extractives from bamboo most effectively. Therefore, the micro-structure of FJS-extracted and untreated bamboo strips (C) were observed using SEM, as shown in Fig. 4.

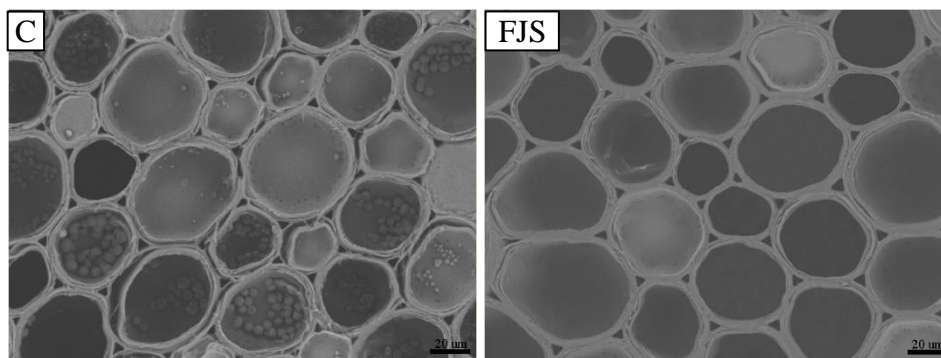


Fig. 4. SEM image of untreated and FJS extracted bamboo strips

The extraction did not alter the cell morphology of bamboo strips. However, in untreated specimens, the lumen of some cells was packed with round particles, while other cells had the same particles at lower densities. Those particles are likely starch in bamboo (Fan 2010), which is an important nutrient for mildew growth and a major reason for mildew-infection in bamboo; therefore, it is necessary to remove this starch. In

Fig. 4, the specimens extracted using the FJS procedure did not have any round particles, suggesting effective removal of nutrients *via* the FJS extraction procedure. The extraction eliminated the required nutrients for mildew growth and thus effectively improved anti-mould properties of bamboo.

### Impacts of Different Extraction Procedures on Anti-mould Properties

#### *Impacts of different extraction procedures on the resistance against *Penicillium citrinum**

Anti-mould tests were performed using *Penicillium citrinum* on untreated specimens (C) or specimens treated with the FJ, FS, SJ, or FJS procedures. The results are shown in Fig. 5. Specimens of the control group (C) were fully covered by *P. citrinum* at eight days after inoculation, with the infection ratio reaching 100%, suggesting that untreated specimens had no resistance against *P. citrinum*. Specimens extracted following the FJ, FS, and SJ procedures presented an average infection ratio of 75%, suggesting that those treatments provided some protection against *P. citrinum*, but the resistance was weak. In contrast, specimens extracted with the FJS procedure presented an infection ratio of 0%, suggesting that FJS extraction provided good protection against *P. citrinum* and created optimum anti-mould properties. These results suggest that FJ, FS, SJ, and FJS extractions improved the anti-mould properties of bamboo to different extents and that FJS extraction significantly promoted bamboo anti-mould properties against *P. citrinum*. The extraction treatments removed large amounts of starch, soluble saccharides, and aliphatics from bamboo strips, which are required for mildew growth.

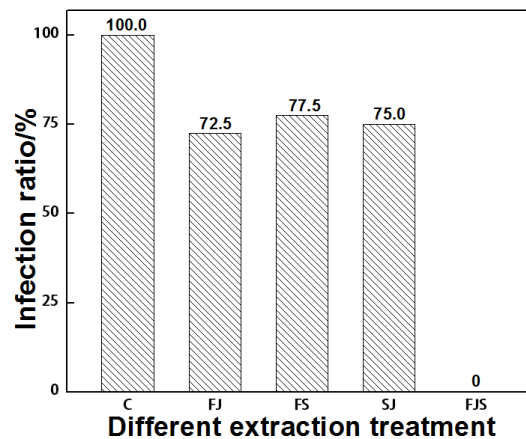
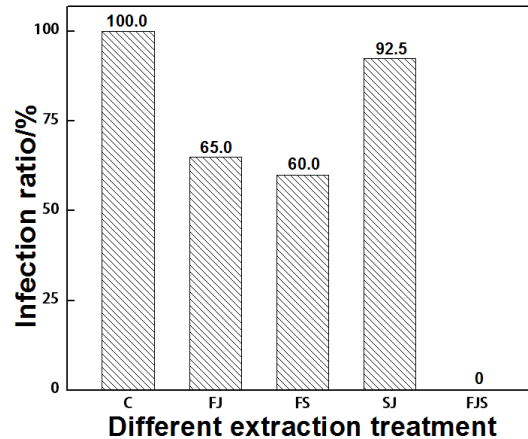


Fig. 5. Infection ratio of *Penicillium citrinum* on untreated or extracted bamboo strips

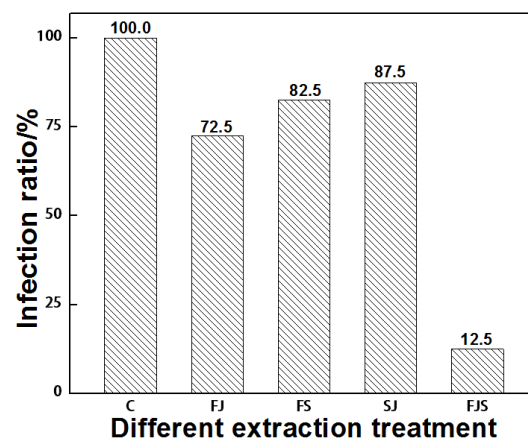
#### *Impacts of different extraction procedures on the resistance against *Trichoderma viride**

Anti-mould tests were performed using *T. viride* on untreated specimens (C) and specimens treated with FJ, FS, SJ, or FJS procedures. The results are shown in Fig. 6.



**Fig. 6.** Infection ratio of *Trichoderma viride* on untreated or extracted bamboo strips

Specimens from the control group (C) were already fully covered by *T. viride* at day six after inoculation, with the infection ratio reaching 100%. This result suggested that untreated specimens had no resistance against *T. viride*. Specimens extracted following the FJ and FS procedures presented infection ratios of 65% and 60%, respectively, suggesting that those treatments provided some protection against *T. viride*, but the resulting resistance was weak. Specimens extracted following the SJ procedure presented a high infection ratio of 92.5%, which was very close to untreated specimens. This suggests that the SJ group specimens had no resistance against *T. viride*. In contrast, specimens extracted with the FJS procedure presented an infection ratio of 0%, suggesting that the FJS extraction provided good protection against *T. viride* and created optimum anti-mould properties. In summary, FJ, FS, and FJS extractions were able to improve the anti-mould properties of bamboo to different extents, and FJS extraction was able to significantly promote the anti-mould properties against *T. viride*.



**Fig. 7.** Infection ratio of *Aspergillus niger* on untreated or extracted bamboo strips

#### *Impacts of different extraction procedures on the resistance against Aspergillus niger*

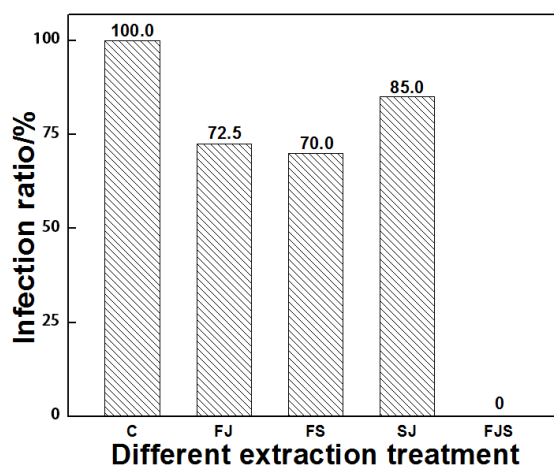
As shown in Fig. 7, specimens of the control group (C) were already fully covered by *A. niger* at day five of inoculation, with the infection ratio reaching 100%, suggesting that untreated specimens had no resistance against *A. niger*. Specimens extracted with the FJ, FS, and SJ procedures presented infection ratios of 72.5%, 82.5%, and 87.5%,



respectively, suggesting that those treatments did not provide efficient protection against *A. niger*. In contrast, specimens extracted following the FJS procedure presented an infection ratio of 12.5%, suggesting that FJS extraction provided decent protection against *A. niger*, but could not completely prevent the onset of infection. In summary, FJ, FS, and SJ extractions did not efficiently improve the anti-mould properties of bamboo strips while FJS extraction notably improved the anti-mould properties of bamboo against *A. niger*.

#### *Impacts of different extraction procedures on the resistance against the mildew mix*

Anti-mould tests were also performed using a mixture of all three kinds of mildew used on untreated specimens (C) and specimens treated with the FJ, FS, SJ, and FJS extraction procedures, and the results are shown in Fig. 8. Specimens of the control group (C) were already fully covered by the mixture of *P. citrinum*, *T. viride*, and *A. niger* at day seven after inoculation, with the infection ratio reaching 100%, suggesting that untreated specimens had no resistance against the mixed mildews. Specimens extracted following the FJ, FS, and SJ procedures presented infection ratios of 72.5%, 70.0%, and 85.0%, respectively, suggesting that those treatments did not provide efficient protection against the mixed mildews. In contrast, specimens extracted following the FJS procedure presented an infection ratio of 0%, suggesting that FJS extraction provided very effective protection against the mildew mixture. In summary, FJ, FS, and SJ extractions did not efficiently improve the anti-mould properties of bamboo strips, while FJS extraction markedly improved the anti-mould properties of bamboo against the mixed mildews.



**Fig. 8.** Infection ratio of mixed mildew on untreated or extracted bamboo strips

**Table 1.** Anti-Mould Efficiency of Bamboo Strips Extracted *via* Different Procedures

Extraction Methods	C	FJ	FS	SJ	FJS
Anti-mould Efficiency (%)	0	29.4	27.5	15.0	96.9

#### **Effects of Different Extraction Procedures on Anti-Mould Efficiency**

The anti-mould efficiencies of untreated specimens (C) and specimens treated with the FJ, FS, SJ, and FJS extraction procedures are listed in Table 1. Untreated



specimens presented an anti-mould efficiency of 0%, indicating no resistance against mildews. Specimens extracted following the FJ, FS, and SJ procedures presented anti-mould efficiencies of 29.4%, 27.5%, and 15.0%, respectively, suggesting that those treatments provided some protection against mildews, but the protections were not sufficiently effective. However, the FJS extraction procedure yielded anti-mould efficiency as high as 96.9%, suggesting that the FJS treatment was able to provide very effective protection against *P. citrinum*, *T. viride*, *A. niger*, and the mixture of these mildews. In other words, those specimens had very good anti-mould properties. Therefore, the FJS extraction procedure was able to greatly improve the anti-mould properties of bamboo strips.

### Mildew Growth on the Bamboo Strip Surface

As addressed above, the FJ, FS, and SJ extraction procedures did not efficiently improve the anti-mould properties of specimens compared to untreated strips (C); therefore, the growth of *P. citrinum*, *T. viride*, *A. niger*, and the mixed mildews were only examined on untreated (C) and FJS-treated specimens (Figs. 9 and 10).



a. *Penicillium citrinum* b. *Trichoderma viride* c. *Aspergillus niger* d. mixture of mildews

**Fig. 9.** Mildew growth on untreated bamboo strips



a. *Penicillium citrinum* b. *Trichoderma viride* c. *Aspergillus niger* d. mixture of mildews

**Fig. 10.** Mildew growth on FJS-extracted bamboo strips

As shown in Fig. 9, untreated bamboo strips were completely covered by *P. citrinum*, *T. viride*, *A. niger*, and the mildew mixture. This complete coverage translates to an infection ratio of 100% and suggests that untreated strips had no resistance to mildew growth. Additionally, all infected bamboo strips were stained by the mildews and lost their natural color. Among them, the black mildew-stain was usually very difficult to remove, completely devaluing the bamboo strips. Consequently, anti-mould treatments of bamboo strips are critical and necessary.

In Fig. 10, FJS-extracted bamboo strips had hardly any mildew growth, and even if they were infected, the average infection ratio was below 3.2%. In addition, the bamboo strips largely retained their original color. These results suggested that the FJS

extraction procedure provided very good protection against *P. citrinum*, *T. viride*, *A. niger*, and mixed mildews and that FJS-extracted bamboo strips had markedly increased anti-mould properties.

## CONCLUSIONS

1. Sequential extraction with the tested procedures did not alter the crystalline structure of bamboo strips. Bamboo strips of groups of FJ, FS, SJ, and FJS presented an increasing trend of hemicellulose degradation and a decreasing trend of the amount of free hydroxyl-groups. These chemical changes caused a decreasing trend of hygroscopicity, which eliminated the moisture required for mildew-growth and resulted in increased anti-mould properties.
2. The sequential extraction procedure (FJS) resulted in migration or elimination of aliphatics and soluble saccharides in the bamboo strips and thus eliminated the nutrients required for mildew growth and improved the anti-mould properties of bamboo strips.
3. Untreated bamboo strips were completely covered by mildews and showed an infection ratio of 100%, suggesting no resistance to mildew growth. The FJ, FS, SJ, and FJS procedures improved the resistance of bamboo strips against *P. citrinum*, *T. viride*, *A. niger*, and a mixture of these mildews to different extents. Furthermore, the FJS extraction procedure resulted in a mildew-prevention efficiency of 96.9%, suggesting optimum anti-mould properties.

## ACKNOWLEDGEMENTS

The authors appreciate the financial support from the National Natural Science Foundation of China (No. 31370568), the CAS Key Laboratory of Engineering Plastics, Institute of Chemistry, the Public Welfare Technology Application Research Project of Zhejiang Province (No. 2017C33029 & 2017C33113), and the Scientific Research Foundation of Zhejiang Agriculture and Forestry University (No. 2013FR088).

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Article submitted: December 8, 2017; Peer revision completed: February 2, 2018;

Revisions accepted: February 13, 2018; Published: February 14, 2018.

DOI: 10.15376/biores.13.2.2658-2669