Preparation and Characterization of Bamboo Strips Impregnation Treated by Silver-Loaded Thermo-Sensitive Nanogels

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Silver-loaded thermo-sensitive nanogels (STSNGs), having a pH value of 6.8, were used as an anti-fungal agent at ambient temperature. To determine the optimal impregnation process, bamboo strips were infused with STSNG by air- and vacuum-pressurization. Scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and X-ray diffraction were employed to characterize the properties and morphology of the resulting impregnated bamboo strips. The results showed that the loading dosage of bamboo strips increased with either prolonged impregnation treatment time, increasing hybrid nanogels concentration, or increasing the intensity of vacuum and pressure. Vacuum-pressurized impregnation remarkably improved the dosage of the hybrid nanogels in the bamboo strips. An increase in the loading dosage resulted in an increase in Ag content. The optimum parameters of impregnation treatment were as follows: 90 min impregnation treatment time, 0.90 wt.% concentration, and 0.5 MPa applied pressure. SEM observations revealed that the STSNGs were successfully saturated in bamboo cell cavities or covered on the cell walls. The results of the mildew proof test showed that the STSNGs had a good anti-mildew effect.

Keywords: Bamboo strips; Thermo-sensitive; Silver-loaded hydrogels; Loading dosage; Impregnation process

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INTRODUCTION

Although China has the most abundant bamboo resources and the highest bamboo yield, the available bamboo output is far less than the original yield due to fungal contamination, weakening its natural appearance and utility (Jiang 2007; Zhang 2007; Zhao *et al.* 2010; Yu *et al.* 2016). Substantial research efforts have been made to reduce the possibility of fungal infection using various anti-fungal agents (Sun *et al.* 2012; Guan *et al.* 2013; Cheng *et al.* 2013; Huang *et al.* 2014; Nguyen and Kim 2015). However, the service life of these existing anti-fungal bamboo products is barely one year when installed outdoors. As such, the development of a novel anti-mildew treatment technology with long-term effectiveness is imperative (Sun *et al.* 004). Thermo-sensitive nanogel exhibits a low critical transition temperature (LCST) (Peng *et al.* 2013). The anti-fungal agent embedded in this nanogel can be released when the surrounding temperature is lower than LCST; otherwise, it is sealed in the nanogel. This switching mode effectively achieves the sustained release of the anti-fungal agent, increasing the effective service life. For a typical example, the LCST of poly (N- isopropyl acrylamide) (PNIPAM) is approximately 32 °C (Kim *et al.* 2015; Wei *et al.* 2015), which is the optimal temperature for fungal growth (25 °C to 30 °C) (Wu and Weng 2000). Because the silver-loaded nanogels usually exhibit a LCST, a beneficial effect is expected only in the temperature range from 30 to 32 °C. The drying of bamboo did not influence the LCST; therefore the PNIPAM nanogel contained in the anti-fungal agent may confer a long-term anti-fungal character to bamboo.

Several researchers have focused on the PNIPAM thermo-sensitive nanogel (Khan *et al.* 2013). Khan *et al.* (2013) prepared Ag/P(NIPAM-co-MAA) nanogel and demonstrated the rapid degradation of nitrophenol. The Ag/PNIPAM nanogel prepared by Zafar *et al.* (2014) could be used to provide anti-fungal activity to textile fabrics. Another gel, Ag/P(NIPAM-co-IA) composite hydrogel synthesized by Jiang *et al.* (2016), presented excellent thermo-sensitive and anti-fungal properties. A pH- and thermo-sensitive Ag/P(NIPAM-co-IA) gel with an anti-fungal property was obtained by Spasojević *et al.* (2015). These anti-fungal gels are widely used in medicine for drug sustained release, surgical dressing, and tissue engineering (Wu *et al*. 2014; Kim *et al.* 2015; Zelikin *et al.* 2016; Zhou *et al.* 2016). Limited research has been done to apply the Ag/PNIPAM gel or modified Ag/PNIPAM gel in bamboo anti-fungal products. Therefore, the aim of this study is to investigate the process of impregnation treatment of silver-loaded thermo-sensitive nanogel in bamboo strips, and to explore the properties of the resultant bamboo strips. Considering the fact that the nano-silvers have stronger inhibiting and killing action on fungi than the currently used anti-fungicide agents, it has the potential to provide bamboo products with long-term anti-fungal properties. The preparation of STSNGs was reported in our previous work, and application in the anti-mildew of bamboo strips with STSNGs (Wei *et al.* 2016a, b, c, d).

EXPERIMENTAL

Materials

Moso bamboo strips (50 × 20 × 5 mm^3, L × W × T, MC: ~10%) without knots were purchased from Zhejiang Yongyu Bamboo Industry Limited by Share Ltd, Huzhou, China.

N-isopropyl acrylamide (NIPAM, analytical grade) was supplied from TCI Co., Ltd (Shanghai, China). N,N'-methylenebisacrylamide (MBA), potassium persulfate ($K_2S_2O_8$, i.e. KPS), N,N,N',N'-tetramethylethylenediamine (TMEDA), silver nitrate ($AgNO_3$), acrylic acid (AAc), and sodium borohydride ($NaBH_4$) are of all analytical grade, and were purchased from Aladdin (Shanghai, China), and these chemical reagents were used as received without further purification. Deionized water was used throughout the experiment.

Test strains for the three fungal strains *Penicillium citrinum*, *Trichoderma viride*, and *Aspergillus niger* were taken from the Department of Microbiology of Zhejiang Agriculture and Forestry University. The strains obtained from the Department of Microbiology, which were directly isolated from natural bamboo mildew, were purified several times.

Methods

Preparation of the silver-loaded thermo-sensitive nanogel

Silver-loaded thermo-sensitive nanogel was made by free radical polymerization. At ambient temperature and normal pressure, a three-necked flask with deionized water (100 mL) under nitrogen was placed in the water bath temperature always at 25 °C. NIPAM, AAc, MBA, and TEMED were added into the deionized water and were continuously stirred until completely dissolved. Silver nitrate solution was gradually added to the mixture without light until the end of reaction system, and then stirred well for an additional 30 min. KPS solution was dropped into the mixture to initiate free radical polymerization without light for 6 h until a transparent mixture was obtained. The resulting gel with silver was finally achieved by adding a $NaBH_4$ solution with a tan color, and the whole reaction was in a sealed state. The gel had a solid content of 0.90 wt.% (initial concentration), with 0.064 wt.% composed of Ag in the whole mixture, the pH value of the solution system was 6.8, and the relative viscosity was 2.0.

Preparation of bamboo strips impregnated by silver-loaded thermo-sensitive nanogel

Air-pressured impregnation method: Bamboo strips were impregnated in the silver-loaded thermo-sensitive nanogel with different concentrations (0.06 wt.%, 0.18 wt.%, 0.45 wt.%, 0.90 wt.%, and 1.20 wt.%) and impregnation treatment times (15 min, 30 min, 60 min, 90 min, and 120 min) under normal temperature and air pressure.

The vacuum-pressured impregnation method was based on air-pressured impregnation method and the addition of various pressures. First, bamboo strips were sealed in the pressurized tank, and the air inside it was then evacuated. When the vacuum reached 0.085 MPa and was maintained for 30 min, the STSNGs were sucked into the tank under the action of vacuum. After the completion of the filling, the valve and vacuum pump were switched off and the tank was held for 10 min. Finally, the pressure values of 0.1 MPa, 0.3 MPa, 0.5 MPa, and 0.6 MPa were successively applied to treat bamboo strips through the impregnation.

The loading dosage of bamboo strips was calculated as follows,

$$\textit{Loading dosage}\ (\%) = \frac{m_1 - m_0}{m_2} * w * 100\% \tag{1}$$

where m_0 is the mass before the impregnation process, m_1 is the mass of the dip treatment was well, m_2 is the mass of drying to 10% of the moisture content after impregnation, and w is the concentration of silver-loaded thermo-sensitive nanogel.

Morphology

To determine the distribution of nanogel in the bamboo strips, the ultra-microstructure morphology of the resulting bamboo strips was imaged by SEM (SS-550, Shimadzu, Kyoto, Japan) with an accelerating voltage of 5 kV.

EDX analysis

A FESEM device (SIRION-100, FEI, Hillsboro, OR, USA) equipped with EDX (Genesis 4000, AMETEK, Newark, DE, USA) was used to perform the content analysis of Ag in impregnated bamboo strips with an accelerating voltage of 25 kV.

XRD analysis

X-ray powder diffraction (XRD) patterns were investigated to confirm the composition of BS-STSNG by using an XRD6000 X-ray diffractometer (Shimadzu) with

Cu Kα radiation at 40 kV and 30 mA. X-ray diffraction data were collected from $2\theta = 5°$ to 80° at a scanning rate of $0.02°\cdot s^{-1}$.

Application of indoor comprehensive anti-mildew test

This was done according to the Chinese standard: GB/T 18261-2013 "Test method for anti-mildew agents in controlling wood mould and stain fungi" (The State Bureau of Quality and Technical Supervision. 2013). The indoor anti-mildew test was carried out under the conditions of 28±2 °C and relative humidity 85±5 %. The growth of three moulds, *Penicillium citrinum*, *Trichoderma viride* and *Aspergillus niger* on the bamboo strips was checked every day and the observations of the attack were recorded properly. At the end of the 28-day experimental period of culture, the observations and analyses were made, and photos of the bamboo strips taken.

RESULTS AND DISCUSSION

Effect of Impregnation Treatment Time on Loading Dosage of Bamboo Strips

Figure 1 exhibits the effect of impregnation treatment times (15 min, 30 min, 60 min, 90 min, and 120 min) on the loading dosage of bamboo strips by impregnating them in STSNGs with the initial concentration of 0.90 wt.% under normal temperature and pressure. The loading dosage of bamboo strips increased with prolonged impregnated treatment time; a noteworthy enhancement was observed when the treatment time was less than 90 min. A gradual increase was seen as impregnated treatment time continued past 90 min, and the plots tended to plateau, due to the fact that cavities near the surfaces of bamboo strips were easily filled with STSNGs at the initial stage of impregnation.

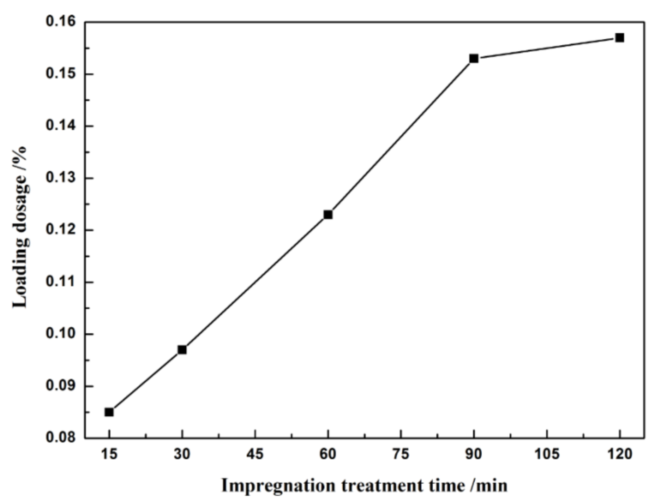

Fig. 1. The effect of impregnation treatment time on the loading dosage of bamboo strips

When the immersion time was prolonged, these cavities became saturated. Moreover, since it was difficult for the STSNGs to permeate the inner cavities of the bamboo, the loading dosage value began to level off after the impregnation treatment for 90 min. For example, the loading dosage of bamboo strips with 120 min treatment time increased only 2.6 wt.% compared with the loading dosage of bamboo strips with 90 min treatment time. As a result, an impregnation treatment time of over 90 min barely had an influence on the loading dosage of bamboo strips, and as such, could be ignored. The optimal impregnation treatment time was 90 min.

The Influence of Concentration on Loading Dosage of Bamboo Strips

At ambient temperature and air pressure, the initial concentration of 0.90 wt.% STSNGs solution was diluted into multiple concentrations of 0.06 wt.%, 0.18 wt.%, and 0.45 wt.%. In addition, according to the selected proportions, STSNGs with the concentration of 1.20 wt.% was synthesized. Then the bamboo strips were impregnated in the STSNGs with various concentrations of 0.06 wt.%, 0.18 wt.%, 0.45 wt.%, 0.90 wt.%, and 1.20 wt.% for 90 min. The resulting loading dosage plot is displayed in Fig. 2.

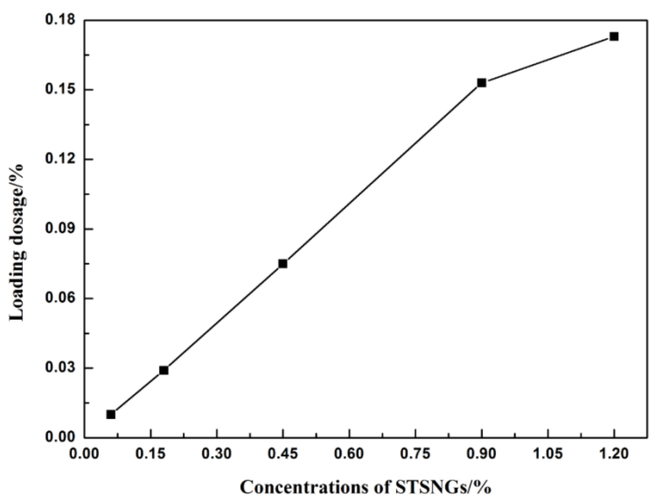

Fig. 2. Influence of concentration on loading dosage of bamboo strips

The loading dosage of bamboo strips rapidly increased with increasing concentration. The loading dosage was 0.153% with a concentration of 0.90%. It was 15.3 times, 5.3 times, and 2 times as high as those of samples with concentrations of 0.06 wt.%, 0.18 wt.%, and 0.45 wt.%, respectively. This demonstrated that the concentrated STSNGs increased the content of silver of bamboo strips, which was beneficial to enhancement of the anti-fungal property. However, the higher the solid content of the STSNGs, the higher the viscosity and thus the poorer flow ability. As a result, the impregnation of bamboo strips became difficult and the loading dosage thus declined. Experimental results revealed that the loading dosage of bamboo strips that was impregnated with the concentration of the STSNG of 1.20 wt.%, was slightly increased by 11.1 wt.% relative to the one concentration of 0.90 wt.%. Thus, the STSNGs having too higher concentration were not suitable to impregnation use of bamboo strips. In this work, 0.90 wt.% was chosen as the appropriate concentration of STSNGSs.

Effect of Vacuum Pressurization on Loading Dosage of Bamboo Strips

In air-pressured impregnation, the loading dosage of bamboo strips was not able to reach a 0.16 wt.% level. This low loading dosage value could not control the release of the anti-fungal agent for a long-term service life. Therefore, vacuum-pressured impregnation treatment was employed to add the loading dosage with pressures of 0.1

MPa, 0.3 MPa, 0.5 MPa, and 0.6 MPa for 90 min treatment time and the initial concentration of 0.90 wt.% STSNGs. The results are shown in Fig. 3.

An increasing loading dosage of bamboo strips was obtained with gradually increasing vacuum pressure. A rapid improvement added loading dosage could be reached by a vacuum pressure range of 0.1 MPa to 0.5 MPa. The dosage (0.5 MPa) was 1.3 times and 1.1 times as high as dosages with 0.1 MPa and 0.3 MPa, respectively. A slightly increased loading dosage value was obtained under 0.5 MPa vacuum pressure, with an increase of only about 1.0 wt.%.

As a result, 0.5 MPa was the optimal parameter. This resulting loading dosage was 3.9 times higher than that of the bamboo strips treated by air-pressed impregnation, illustrating that vacuum-pressured impregnation could increase the loading dosage of bamboo strips. This was due to the fact that the vacuum pressurization expelled the internal air with low cell space, resulting in low resistance to the entrance of STSNGs and an enhancement for impregnation and absorption. High loading dosage led to a guarantee of the long-term release of anti-fungal agent.

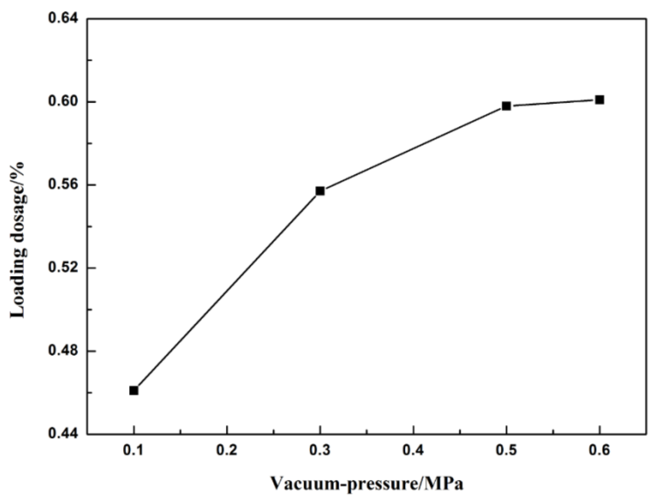

Fig. 3. The relationship between vacuum pressurization and loading dosage of bamboo strips

In summary, at the same concentration of 0.90 wt.% and treatment time of 90 min, the vacuum-pressured impregnation process of bamboo strips with STSNGSs at a pressure of 0.5 MPa was better than the air-pressured method.

Ultra-microstructure of Impregnated Bamboo Strips

Figure 4 shows the morphology of an original bamboo strip with drying to the moisture content 10%, a bamboo strip treated by air-pressured impregnation, and a bamboo strip treated by vacuum-pressured impregnation. Both impregnation treatments did not change the microstructure of bamboo strips, though they shrunk the cell void spaces, with the void spaces even vanishing when treated by vacuum-pressured impregnation. The main reason for this was the fact that the bamboo cells were partially/totally filled by STSNGs. Vacuum-pressured impregnation led to a higher coverage in the cell cavity and cell wall, providing further evidence for the high efficiency of vacuum-pressured impregnation.

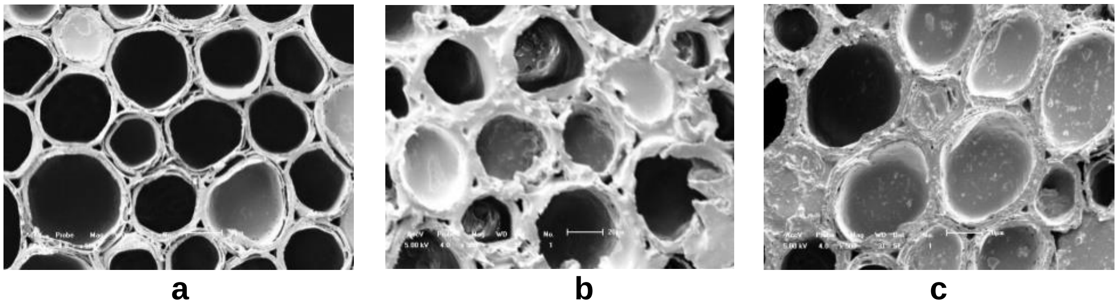

Fig. 4. SEM images of impregnated bamboo strips with various treatment methods. a) Untreated; b) Air-pressured impregnation: concentration 0.90 wt.%, treatment time 90 min; c) Vacuum-pressured impregnation: concentration 0.90 wt.%, treatment time 90 min, and pressure 0.5 MPa

EDX Analysis of Impregnated Bamboo Strips

The EDX patterns of bamboo strips with different treatment methods are shown in Fig. 5. The feature peaks of C and O appeared in the EDX patterns.

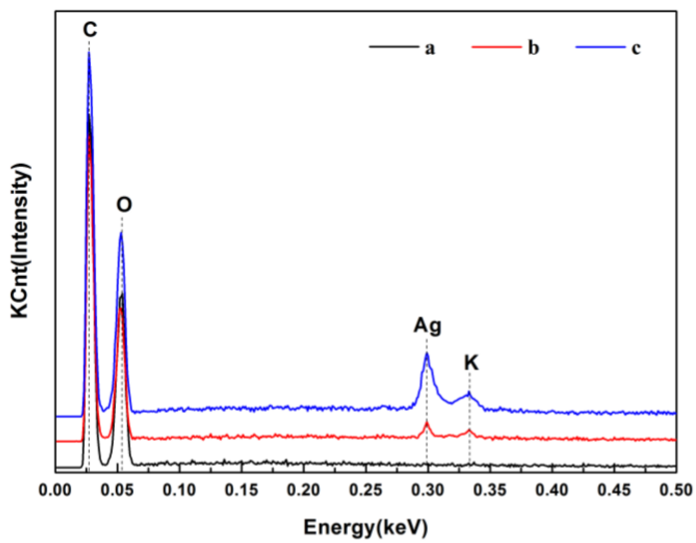

Fig. 5. The EDX patterns of bamboo strips with different treatment methods. a) Untreated; b) Air-pressured impregnation: concentration 0.90 wt.%, treatment time 90 min; c) Vacuum-pressured impregnation: concentration 0.90 wt.%, treatment time 90 min, and pressure 0.5 MPa

Another peak representing Ag was observed in the EDX of impregnation-treated bamboo strips, implying that Ag was loaded in the bamboo strips. The content of Ag loaded by air pressure was 0.86 wt.%, almost one-sixth as high as the content loaded by vacuum pressurization, 5.17 wt.%. This illustrated that vacuum pressurization improved the loading dosage with higher efficiency, leading to the presence of more Ag added to the bamboo strips. Due to $K_2S_2O_8$ acting as an initiator of NIMPAM monomer in this case, small amounts of K ions may reside inside the hybrid nanogels. And the content of K element increases with an increase in loading level of the STSNGs. Therefore, the elevated vacuum pressure would result in an increase in the content of K inside the bamboo strips, but this increase was not related to the increased content of Ag in the bamboo strips (Fig. 5 and Table 1).

Table 1. Elemental Analysis of Bamboo Strips with Different Treatment Methods

Samples	C (wt.%)	O (wt.%)	Ag (wt.%)	K (wt.%)
a	62.20	37.80	0	0
b	62.35	36.36	0.86	0.43
c	57.36	36.44	5.17	1.03

Note: a) Untreated; b) Air-pressured impregnation: concentration 0.90 wt.%, treatment time 90 min; c) Vacuum-pressured impregnation: concentration 0.90 wt.%, treatment time 90 min, and pressure 0.5 MPa

XRD Analysis of Impregnated Bamboo Strips

The similar XRD patterns of the different treated bamboo strips and original bamboo strips are presented in Fig. 6.

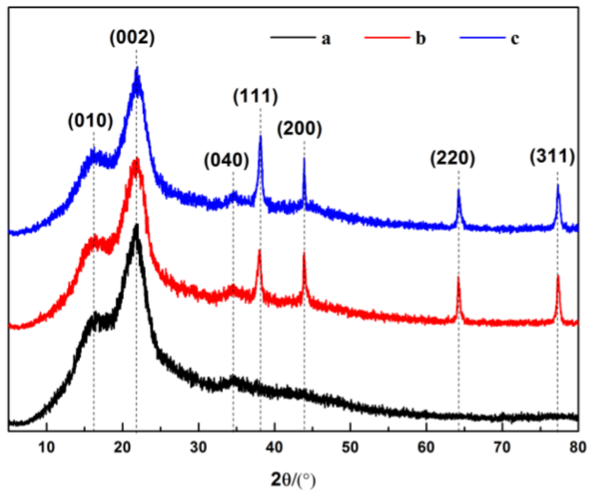

Fig. 6. The XRD patterns of bamboo strips with various treatment methods. a) Untreated; b) Air-pressured impregnation: concentration 0.90 wt.%, treatment time 90 min; c) Vacuum-pressured impregnation: concentration 0.90 wt.%, treatment time 90 min, and pressure 0.5 MPa

The diffraction peaks, *i.e.* (010), (002), and (040), corresponded to the cellulose I crystal without any movement, implying that the impregnation treatment maintained the crystalline structure, and a physical combination occurred throughout the entire process, and not formation of chemical bonds. In addition, four diffraction peaks at 38.1°, 44.3°, 64.4°, and 77.6°, namely (111), (200), (220), and (311) corresponded to the silver element (Fan *et al.* 2009; Krstić *et al.* 2014), which further indicates the existence of STSNGs in the bamboo strips.

Application in the Comprehensive Mold Proof of Bamboo Strips with STSNGs

The above-mentioned impregnation process was used to treat the bamboo strips with STSNGs, while the untreated bamboo strips as control groups were impregnated with deionized water for 90 min. Finally, indoor anti-mildew test was achieved after 28 days, and the experimental results are shown in Fig. 7.

Figure 7a shows that the surface of the untreated bamboo strips was covered with clearly visible *Penicillium citrinum*, *Trichoderma viride*, and *Aspergillus niger*. The damage value reached 4.0 (the sample surface infection area more than 75%), therefore the bamboo strips did not have a mildew-proof effect. In addition, the untreated bamboo strips lost their natural color. The black color due to the mildew was very difficult to remove, resulting in the loss of use value of the bamboo, as well as the necessity to apply mold treatment. It is shown in Figs. 7b and 7c that the bamboo strips of STSNGs were impregnated with air-pressure, and there was only a mild fungal infection, such that the value of the damage reached 1.0 (the sample surface infection area less than 25%). However, the vacuum-pressure treatment of STSNGs of bamboo prevented fungal infection, such that the value of the damage reached 0 (the sample surface was free of mycelium). Thus, it can be seen that the bamboo strips impregnated with STSNGs and exposed to *Penicillium citrinum*, *Trichoderma viride*, and *Aspergillus niger*, which are three kinds of bamboo fungi, had better control effect, and the effect of vacuum-pressured impregnation treatment was much better than that of air-pressured impregnation. Therefore, the vacuum-pressured impregnation was judged to be a better anti-mildew method for treatment bamboo strips with STSNGs.

a b c

Fig. 7. The mildew-proof effect after 28 days of bamboo strips with a) Untreated; b) air-pressured impregnation: concentration 0.90 wt.%, treatment time 90 min; c) vacuum-pressured impregnation: concentration 0.90 wt.%, treatment time 90 min, and pressure 0.5 MPa

CONCLUSIONS

1. In air pressure impregnation treatment, the loading dosage of silver-loaded thermo-sensitive nanogel on bamboo strips increased with increasing impregnation treatment time and increasing nanogel concentration. The concentration of 0.90 wt.% and the impregnation treatment time of 90 min resulted in a high loading dosage of the bamboo strips.
2. In vacuum pressurization, increasing pressure improved the loading dosage of the bamboo strips. Compared with the air pressurization impregnation, impregnation with vacuum pressurization significantly enhanced the loading dosage of the bamboo strips. The optimal pressure was 0.5 MPa.
3. The silver-loaded thermo-sensitive gel penetrated the interior of the bamboo strips, filled in cell cavities, and covered the cell walls without any change in the bamboo microstructure.
4. The increasing loading dosage of the bamboo strips led to an increase in Ag, providing a protective, long-term anti-fungal effect.

5. Bamboo strips impregnated with STSNGs and exposed to three kinds of bamboo fungi, *Penicillium citrinum*, *Trichoderma viride* and *Aspergillus niger*, exhibited better control effect than untreated bamboo strips, and the vacuum-pressured impregnation process proved to be a better anti-mildew method.

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