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The bactericidal and mildew-proof activity of a TiO_2–chitosan composite

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ABSTRACT

The current study combines the TiO_2 photo-catalysis and the bactericidal activity of chitosan (CS), incorporated into a novel bactericidal and mildew-proof fabric (BMF) through immobilization of TiO_2–CS composite on cotton fibers. The optimum preparation conditions of BMF were determined. The bactericidal activities of samples were evaluated by the inactivation of three strains, *Escherichia coli*, *Staphylococcus aureus* and *A. niger*. The results showed that the bactericidal yields of BMF for *E. coli*, *S. aureus* and *A. niger* reached respectively 99.9%, 99.9% and 97.4% after 12 h visible light irradiation. The long-lasting bactericidal ability of BMF is stable due to most of *E. coli*, *S. aureus* and *A. niger* being completely killed or broken by the micrograph of cell debris. The UV–vis transmission spectra show a strong absorption that spans the visible light spectral region. A possible explanation of the photo-catalytic action of BMF is proposed.

1. Introduction

During the past decades, human health was seriously threatened by environmental pollution, especially indoor microbiological contamination. The Chinese pandemic of "SARS" in 2003 and of "Influenza" in 2009 caused world-wide panic. The statistics reveal that the total number of deaths caused by bacterial infection exceeds 17 million, about 1/3rd of world-wide deaths [1]. It is hence urgent to seek develop and improve green bactericidal material capable to eliminate environmentally spread bacterial infections. Previous research showed that CS and nano-TiO_2 were green bactericidal materials, and might be promising candidates in solving this problem [2–5].

CS is obtained by N-deacetylation of chitin, the second most abundant natural polysaccharide [6]. Due to its good biocompatibility [6,7], CS was widely applied in the field of the pharmaceutical [6–8], agriculture, food, nutrition and biomedical industries [9], with additional applications in e.g. wastewater treatment [10]. In recent years, the bactericidal and antifungal activities of CS received extensive attention [10–15]. In aqueous solutions, the cationic amino groups of the CS structure can electrostatically interact with negatively charged molecules such as mucosal membranes on the bacteria cell surface [16]: this alters the cell permeability and results in ceasing intracellular transport or leakage of intracellular biomaterial [17]. Bacteria will be finally inhibited or killed [10]. Literature also reported different bactericidal mechanisms of CS and suggested that the bactericidal action came from a lower pH derived from organic acids rather than from CS [18,19]. Moreover pure CS shows some disadvantages in the application, such as unsatisfactory mechanical properties, severe shrinkage, deformation after drying and higher solubility under acidic condition [20].

Recently, semiconductor photo-catalyst has been widely investigated as bactericidal materials. The organic contamination could be decomposed due to photo-generated holes/electron and reactive oxygen species (ROS) in photochemical reactions, such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\bullet$), which results in an enhanced bactericidal activity of semiconductor photo-catalyst [11,20,21]. Photo-catalytic TiO_2 is cited as especially attractive and is widely applied since chemically stable, non-toxic, inexpensive, and exhibiting a high photo-catalytic efficiency [13,18]. Caballero reported that nano-TiO_2 has deodorant and bactericidal activities in fabrics as photo-catalyst [6]. However, the real efficiency of nano-TiO_2 was reduced by its unstable thermodynamic properties and serious agglomeration. Nano-sized TiO_2 powder was moreover difficult to be recycled, which also limits its commercial application.

To counter-act these drawbacks, several composite materials have recently been prepared by nano-TiO_2 and organic polymers to combine the properties of organic polymers (e.g. flexibility, ductility and dielectric), but also exhibit the particular photo-catalytic properties of inorganic materials [19,20]. CS–TiO_2 and CS–SiO_2 complex films were reported due to their good surface properties and bactericidal activities [21–23]. For a widespread practical application in daily life, it is however needed to improve the catalytic activity in order to achieve effective bactericidal activity of TiO_2.

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The present study therefore investigates the combination of the photo-catalysis characteristics of TiO_2 and the bactericidal ability of CS within a novel bactericidal and mildew-proof fabric (BMF) through immobilization of TiO_2–CS composite on cotton fibers. The preparation process was optimized. The characteristics of BMF were determined by FT-IR and UV–vis spectroscopy. The bactericidal activities of samples were evaluated by inactivation of three strains, *Escherichia coli*, *Staphylococcus aureus* and *A. niger*. The possible mechanisms for the bactericidal activity will be discussed.

2. Experimental

2.1. Materials

CS (degree of deacetylation > 85%) was extracted from shrimp shells and obtained from Jinan Hyderabad bay (China); titanium dioxide (P25, 80% anatase, 20% rutile, 50 m^2/g, primary particle size 25–30 nm) was purchased from Degussa (Germany); analytical grade materials, such as epichlorohydrin (ECH), silane coupling agent (KH550), dodecyl sodium sulfate (SDS), acetic acid, sodium chloride and glucose were purchased from Chemical Reagent Company of Beijing (China). Beef peptone, beef extract powder; yeast extract powder and agar were of biochemical grade. *E. coli* (ATCC 8099), *S. aureus* (ATCC 6538), and *A. niger* (ATCC 16404) were obtained from the American Type Culture Collection. De-ionized water was used in all experiment.

2.2. Preparation of bactericidal and mildew-proof emulsion (BME) and bactericidal and mildew-proof fabric (BMF)

BME was prepared by adding 200 mL 2.5 vol.% acetic acid into three beakers containing respectively 1 g CS, 0.5 g TiO_2, or 1 g CS + 0.5 g TiO_2. The mixed solution was homogeneously dispersed by a SK3300HP ultrasonic vibrator (Kudos, China) for 5 min, followed by the addition of 5 mL 2.5 vol.% epichlorohydrin, 0.01 mol/L SDS and a piece of 50 cm × 50 cm cotton fabric for polymerization during a 15 min ultrasonic vibration. Finally, the treated fabric (BMF) was dried in vacuum for 14 h. The grafting efficiency was calculated as follows:

$$\text{Grafting efficiency (GE\%)} = \left[\frac{W_3 - W_2}{W_1}\right] \times 100 \qquad (1)$$

where W_1, W_2, and W_3 denote the weight of CS/TiO_2/CS–TiO_2, of cotton fabric, and grafted cotton fabric, respectively.

2.3. Characterization

FT-IR spectra analysis: FT-IR spectra of cotton fabric and BMF were determined with a VARIAN-3100 Fourier-transform infrared (FTIR) spectrometer in the range of 4250–500 cm^{-1} (Varian, USA) to monitor the change of chemical bond.

UV–vis spectra: UV–vis spectra were recorded by a Hitachi U-3010 UV–vis spectrophotometer (Hitachi High-Technology Corporation, Tokyo, Japan).

Scanning electron microscopy (*SEM*): Cotton fabric, as such or after treatment to BMF were examined using a S4700 electron microscope (Hitachi, Japan) with a 20 kV/10 μA electron beam.

Microscope picture: Samples containing 10^7 colony formation unit (CFU)/mL *A. niger* in nutrient broth were contacted with BMF and illuminated by a common household light of 20 W, 50 Hz for up to 2 h. Thereafter, 300 μL bacteria suspension was deposited on the slide glass using a micro-syringe, and a drop of 0.2 wt% methylene blue was added to fix bacteria cells for 5 min. The slide glass was examined under a 400× magnification using a DME microscope (Leica, Germany).

Table 1
Property of graft polymerization.

Copolymer	CS	TiO_2	CS–TiO_2
Efficiency of grafting polymerization (%)	86.2	35.5	61.7

Reaction conditions: CS: 1 g; TiO_2: 0.5 g; acetic acid 2.5%; 25 °C; 15 min.

2.4. Bactericidal assay

2.4.1. Test of bactericidal ratio

E. coli (ATCC 8099), *S. aureus* (ATCC 6538), and *A. niger* (ATCC 16404) were used as test organisms. BMF and cotton fabric were dipped into a flask containing 100 mL sterile saline suspension containing a cell concentration of 4.55×10^5 CFU/ml (*E. coli* and *S. aureus*) and 3×10^4 CFU/ml (*A. niger*), respectively. To ensure the adequate contact between bacterial suspension and bactericidal materials, the flasks were shaken on a rotary shaker of 170 rpm and illuminated by a common household light (20 W, 50 Hz) at 37 °C for *E. coli* and *S. aureus*, or at 28 °C (*A. niger*). 0.2 mL bacteria suspension was removed at the fixed time and spread on triplicate nutrient agar plates. After incubated for 24 h, the number of colonies was counted by enumeration of viable organisms. All materials were autoclaved at 121 °C for 20 min to ensure sterility prior to use. The bactericidal ratio was calculated as follows:

$$\text{Bactericidal ratio} = \frac{N_0 - N_t}{N_0} \times 100\% \qquad (2)$$

where N_0 and N_t stand respectively for the total number of colonies before and after reacting with bactericidal material at a given time *t*.

The bactericidal kinetics curves of the bacteria which contacted different bactericidal materials were made according to the numbers of bacteria at different time *t*. *E. coli* was chosen as test microorganism.

2.4.2. Batch test of BMF

Batch tests of the bactericidal ratio were carried out using the same BMF as test sample. Firstly, the bactericidal experiment of BMF was performed using the experiment method of Section 2.4.1. Secondly, BMF was washed using the de-ionized water and finally dried in vacuum for next use.

2.4.3. Long-lasting bactericidal test of BMF

Long-lasting bactericidal tests were performed through extending contact times of BMF with strain suspensions for up to 60 h. The microorganisms were then incubated for 24 h using the experiment method of Section 2.4.1.

3. Results and discussion

3.1. Effect of the preparation process on bactericidal activity of BMF

3.1.1. Graft polymerization property of TiO_2–CS on cotton fibers

The bactericidal and mildew-proof fabric (BMF) was prepared by immobilization of TiO_2–CS complex on cotton fibers using a graft polymerization technology. The grafting efficiency of TiO_2–CS on cotton fabrics is shown in Table 1.

Since the low solubility of nano-TiO_2 powder in acetic acid or de-ionized water is a disadvantage in the graft polymerization, the grafting degree of TiO_2 was below 35.5% as shown in Table 1. Otherwise, the grafting efficiency of CS on cotton fabric reached 86.2% by hydrogen bonding reaction between the functional groups (–NH_2) on the CS structure and hydroxyl groups on the cotton fabric. With the help of the CS, the solubility and compatibility of nano-TiO_2 in emulsion were enhanced. Consequently, the

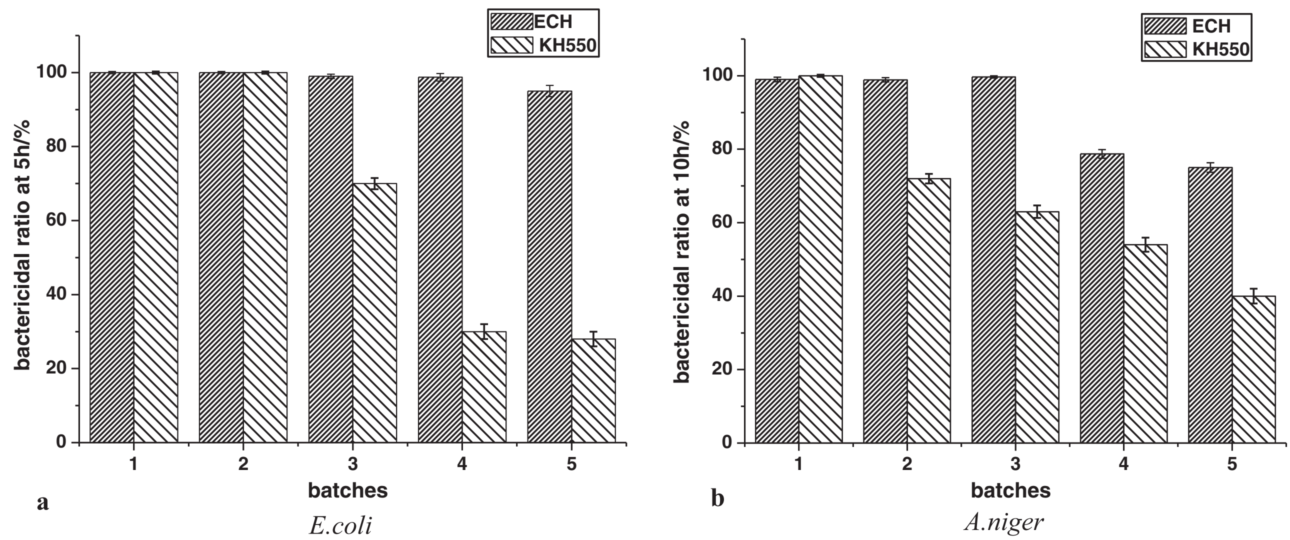

Fig. 1. Effect of crosslinking agents on the bactericidal activity of BMF.

grafting efficiency of TiO_2–CS complex on cotton fibers was raised from 35.5 to 61.7%.

3.1.2. Selection of cross linking agent in the preparation process

Effect of cross linking agent on bactericidal activity of BMF: ECH and KH550 were chosen as cross linking agents in order to improve the grafting efficiency of BME with cotton fabric. As shown in Fig. 1, the bactericidal ratios of BMF with ECH as cross linking agent were better especially after 5 bactericidal text cycles, and reached the three-fold value for *E. coli* and the double value for *A. niger* in comparison with those of BMF with KH550 as cross linking agent, most probably due to the different functional groups on ECH and KH550 structure: ECH with ring oxygen bond has a higher reactivity and maybe easily broken to link with cellulose on the cotton fabric's surface, which led to a higher grafting stability; KH550 on the other hand has amino groups that will compete with amino groups of the CS structure, thus decreasing the bonding force between CS and cotton fabric. The bactericidal activity when using KH550 as cross linking agent decreased quickly after multiple recycle.

Effect of cross linking reaction time on bactericidal activity of BMF: The literature reported that the possible mechanism for bactericidal activity of CS was the cationic amino groups of CS molecules reacting with negative charges on the cell wall and cell membrane, which would disturb the electric stability of the cell structure leading to the inhibition or killing of bacteria [10]. Therefore, the decrease of free amino groups on CS could lead to the decline of bactericidal ability. However the lower grafting loading of TiO_2–CS on cotton fibers through cross linking reaction will decrease bactericidal activity of BMF after multiple recycling.

In BMF preparation, the optimum time of the cross linking reaction was investigated for higher bactericidal activity of BMF. As can be seen in Fig. 2, the bactericidal activity of BMF with ECH as cross linking agent increased from 15 min to 30 min, and then decreased at 60 min. Since the functional groups (–NH_2) on the CS structure were very active, and easily linked with cellulose on the cotton fabric's surface through hydrogen bond, for reaction times below 30 min, it was relatively insignificant to achieve a well-load coating of CS and TiO_2 on the cotton fabric surface; for a longer cross linking time, there were excessive amino groups of the CS molecule linked, which led to the decrease of free amino groups on the CS structure. The bactericidal activity of BMF observably decreased. Therefore, the optimum cross linking reaction time of 30 min was applied in this work.

Effect of cross linking agent concentration on bactericidal activity of BMF: According to the study of cross linking reaction time on the bactericidal activity, an optimum concentration of cross linking agent in the preparation process should exist for obtaining a higher bactericidal activity and a feasible loading efficiency. The bactericidal behaviors of *A. niger* were therefore studied with the ECH concentration of 10%, 5%, 2.5% and 1.25% (v/v).

Fig. 3 shows that the bactericidal ratio was almost up to 100% at ECH concentration of 2.5% (v/v), while the bactericidal activity obviously declined at the concentration below or above 2.5% (v/v). One possible reason was that the cross linking reaction could be weakened or enhanced between the CS or TiO_2 and cellulose, all of them leading to the loss of the bactericidal activity of BMF. So the optimal concentration of cross linking agent of 2.5% (v/v) was selected in this work.

3.1.3. FT-IR spectra analysis

IR spectra of cotton fabric and BMF were obtained with a VARIAN-3100 Fourier-transform infrared (FTIR) spectrometer in the range of 4250–500 cm^{-1}. The absorption characteristic peaks of 1669 and 1706 cm^{-1} were assigned to the carboxyl and ether group on cellulose structure [11]. The characteristic peaks of the single bond of oxygen and titanium and Ti–O–C

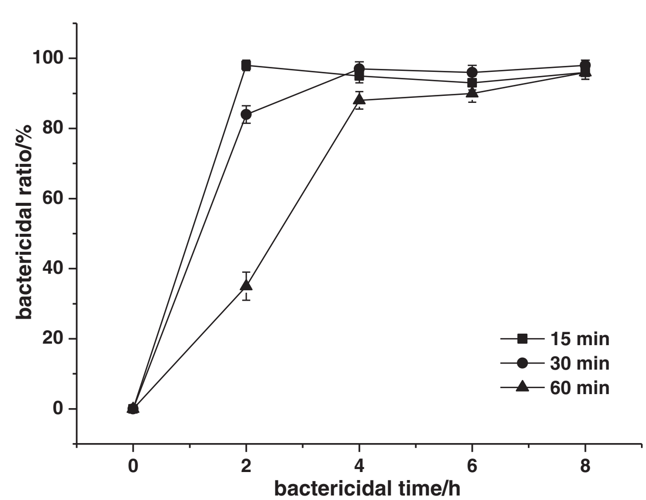

Fig. 2. Effect of crosslinking time on the bactericidal activity of BMF.

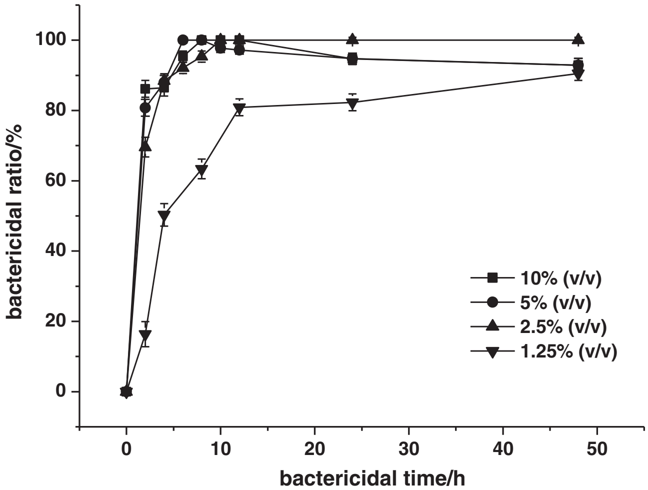

Fig. 3. Effect of crosslinking agent concentration on the bactericidal activity of BMF.

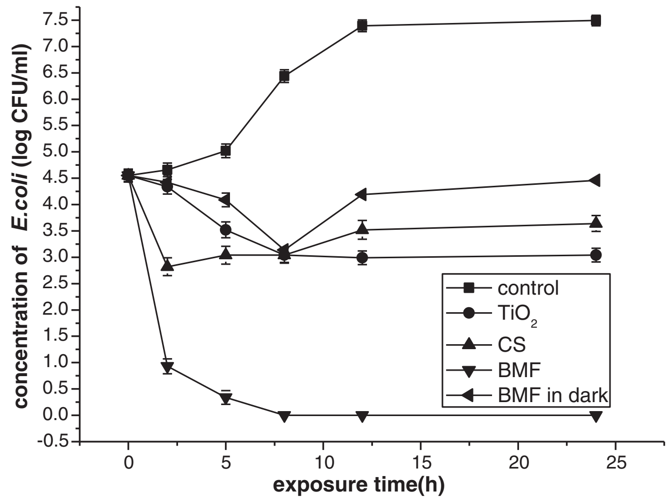

Fig. 6. Bactericidal kinetics of *E. coli* in the presence of CS/TiO_2/BMF.

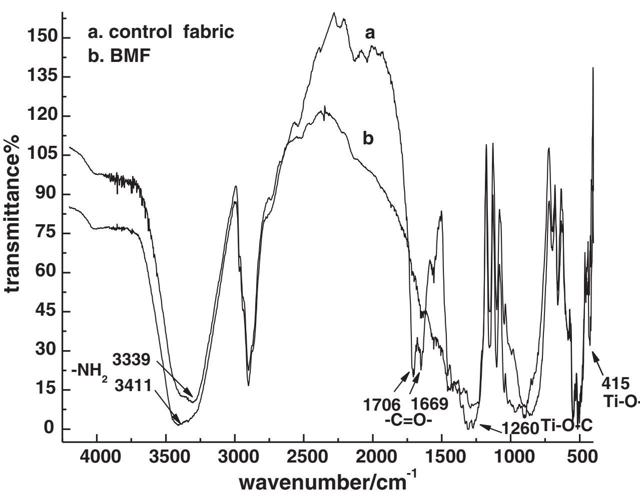

Fig. 4. FT-IR spectra of cotton fabric (a) and,BMF (b).

peak were observed at 415 cm^{-1} [24] and 1260 cm^{-1} in Fig. 4b, respectively. It is noteworthy that due to the formation of hydrogen bonding between CS and cotton fabric, the peak associated with the hydroxyl group of cellulose shifted from 3339 cm^{-1} to 3411 cm^{-1}, while the typical cellulose peak of the cotton fabric in the range of 1238–1726 cm^{-1} disappeared [25]. The results confirmed the grafting reaction between the carboxyl and ether group of cellulose on the cotton fabric, and amine groups on the CS molecule.

3.1.4. Ultraviolet–visible spectroscopy

The UV–vis transmission spectra of TiO_2 powder (curve a), primitive CS emulsion (curve b) and CS–TiO_2 composite emulsion (curve c) are shown in Fig. 5. According to previous experiments [26], the strongest absorption peak of TiO_2 powder appeared at about 272 nm. The strong absorption range at approx. 200–400 nm was in agreement with the lower photo-catalytic ability of TiO_2 powder under visible light irradiation (curve a). As can also be seen for the CS emulsion (curve b), no absorbance occurred from 350 to 800 nm. However for the CS–TiO_2 emulsion (curve c), the absorption values gradually decreased at longer wavelengths, and a wide and strong absorption band was observed at 300–500 nm. The strong absorption edge extended over the visible light spectral region, thus confirming the high bactericidal efficiency of BMF under visible light. The red shift might be caused by the combination between TiO_2 particles and CS through hydrogen bonding [20].

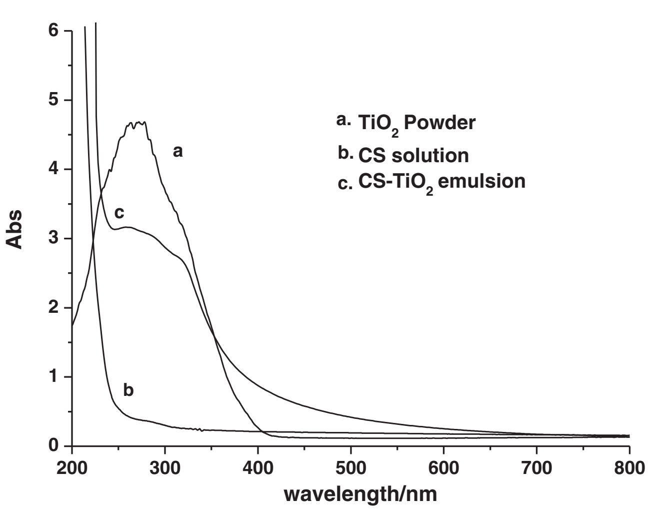

Fig. 5. UV–vis spectra of TiO_2 powder (a), CS solution (b) and CS–TiO_2 emulsion (c).

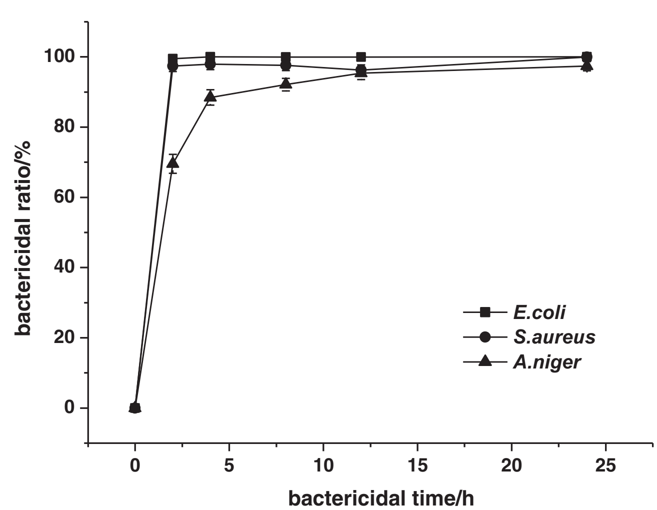

Fig. 7. Bactericidal activity of BMF to *E. coli*, *S. aureus* and *A. niger*.

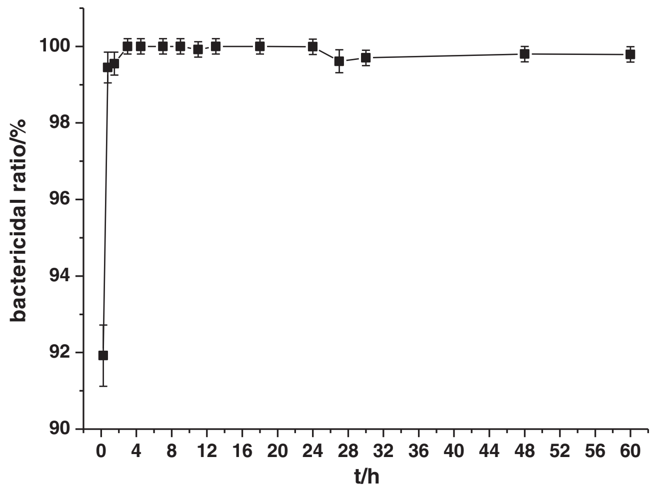

Fig. 8. Long-lasting bactericidal test.

3.2. Study of the bactericidal characteristics and mechanism of BMF

Assessment of dynamic bactericidal activities of BMF: The assessment of dynamic bactericidal activity was carried out by quantitatively measuring the bactericidal activity of BMF. The bactericidal kinetics curve of *E. coli* is shown in Fig. 6. The results showed that both of TiO_2 and CS offered low bactericidal activity for *E. coli*, since pure TiO_2 powder had a poor loading efficiency on cotton fabric (see Table 1), which led to the easy removal of nano-TiO_2 particles from the cotton fabric surface. A weaker bactericidal activity of CS was also displayed in Fig. 6, however in agreement with reported values [18,27]. Simultaneously, the bactericidal activity of BMF under dark conditions was also very low and similar to that of pure CS. The result showed that the photo-catalytic reaction of TiO_2 with the help of the UV irradiation was crucial for enhancing bactericidal activity. Compared to TiO_2 and CS, an outstanding bactericidal activity of BMF was observed. Three reasons might be used to explain this phenomenon. Firstly, the presence of CS increased the compatibility of TiO_2 with cotton fabric; secondly, the coexistence with TiO_2, CS maybe enhanced the bactericidal activity of BMF. Simultaneously the strong absorption edge extended over the visible light spectral region might be one of possible reasons for high bactericidal activity (Fig. 5). Based on the synergy and complementarity of the bactericidal property of CS and the photo-catalytic technology of nano-TiO_2 [12], the bactericidal ability of BMF was significantly strengthened.

Effect of bacteria kinds on bactericidal activity of BMF: Two strains of bacterium (*E. coli* and *S. aureus*) and one strain of fungus (*A. niger*) were chosen as test microorganisms. The bactericidal abilities of BMF on *E. coli*, *S. aureus* and *A. niger* are displayed in Fig. 7. The bactericidal ratios of *E. coli* and *S. aureus* were higher than that of *A. niger*. The reason might be that *A. niger* had a more effective protection mechanism under unfavorable conditions due to mainly its thicker cell wall, reproduction and modes of spore production. Compared to Gram-positive bacteria (*S. aureus*), the bactericidal ratio of Gram-negative bacteria (*E. coli*) was slightly higher due to their thin cell wall and low cross linking degree of peptidoglycan [12,13,18,28]. This showed that the structure of the cell wall could play an important role in the bactericidal activity of BMF [6,7].

Study on long-lasting bactericidal ability of BMF: In this experiment, *E. coli* was chosen as test microorganism. Results revealed a sharp increase at the beginning of the reaction (Fig. 8). The bactericidal rate for *E. coli* reached 98% after 2 h irradiation, and then, the bactericidal ratio exceeded 99% without any decline until 60 h, demonstrating that the bactericidal activity of BMF is attributed to the permanent nature of TiO_2 and CS [5], which indicated that it can thoroughly kill or break all the *E. coli* cells instead of simply inhibiting growth. This is further confirmed in Section 3.3.

3.3. Mechanisms of the bactericidal activity

Based on the coupling of photo-catalysis and physical chemistry reactions, the possible mechanisms for the bactericidal activity of BMF were investigated. *A. niger* was again used as test bacteria. The morphology of the TiO_2–CS coating on the texture surface was observed by SEM.

Compared to pristine cotton fabric (Fig. 9a), loosely deposited particles of about 2–5 μm on the texture surface were observed from Fig. 9b. Nevertheless, Fig. 9c displays a smoother and homogeneous surface of the texture. This revealed that, with the help of CS emulsion, nano-TiO_2 particles formed strong linking-bonds with cotton fabric or CS molecules, and hence were not removed after 5 batch tests (Fig. 1). It also was one of possible reason accounting for long-lasting bactericidal ability of BMF.

The morphology tests of bacteria were further examined by microscope at a 400× magnification after treatment with different bactericidal materials for 2 h. As shown in Fig. 10a, *A. niger* cells kept their morphology after being in contact with pristine fabric during 2 h, while the cell structure deformed to different extents after having been in contact with CS fabric (Fig. 10b) and TiO_2 fabric (Fig. 10c). Cells were nearly broken down to debris after they contacted BMF (Fig. 10d). These results further indicated that the bactericidal ability of BMF was enhanced and the bacteria were thoroughly broken instead of just being inhibited. Choi et al. [29] and Helander et al. [30] reported that the CS molecules could adhere to cell membrane surface by its cationic amino groups ($–NH_3^+$) and penetrate into the living cell through the phosphor-lipid bi-layer,

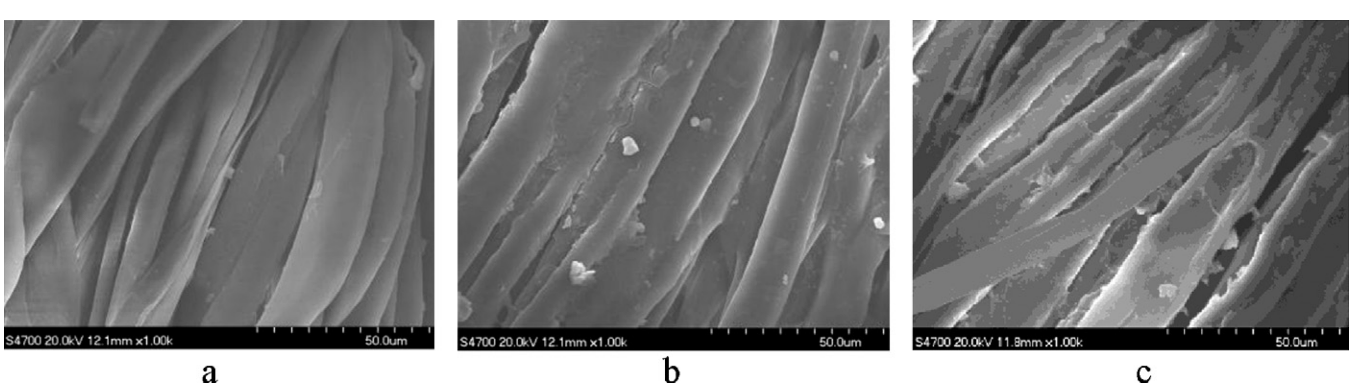

Fig. 9. SEM pictures of cotton fabric (a), BMF (b) and BMF after 10 batch tests (c).

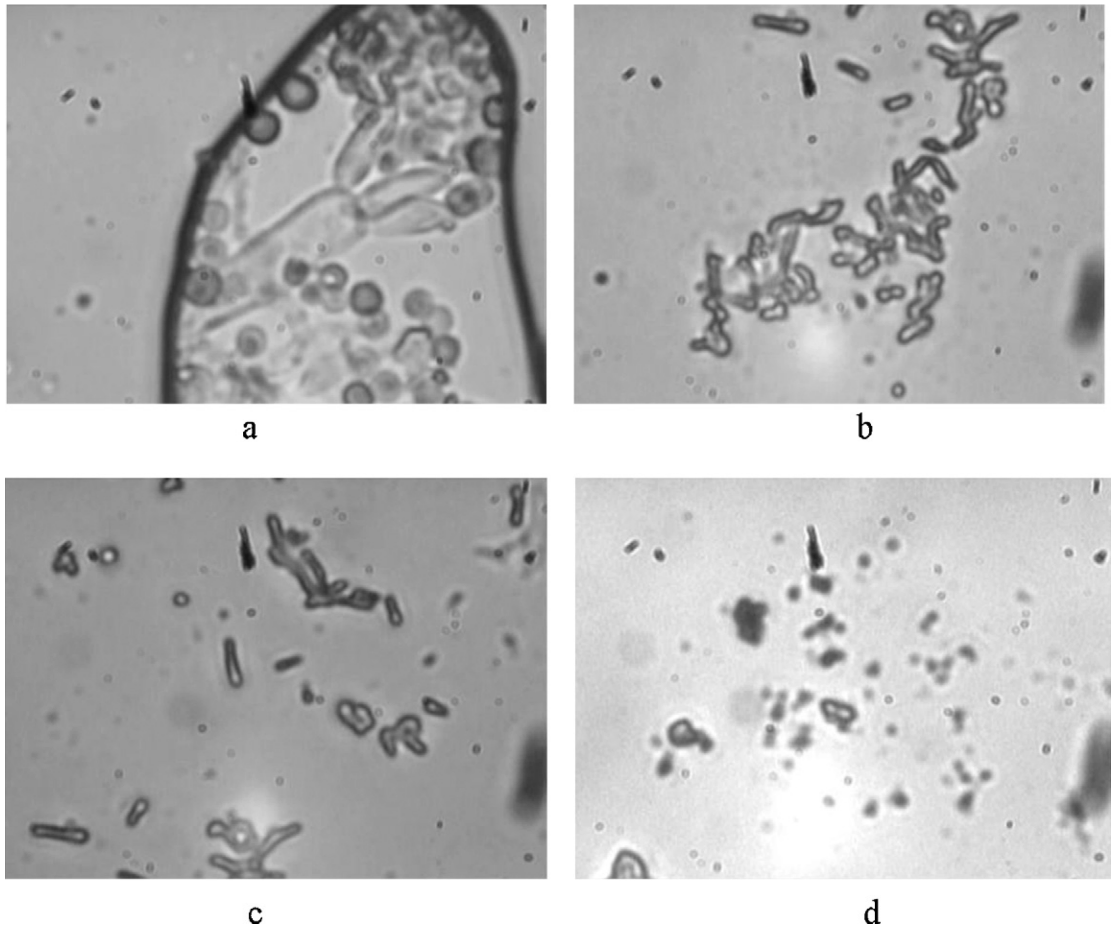

Fig. 10. Microscope pictures of *A. niger* cells exposed to bactericidal fabrics, pristine fabric (a), CS fabric (b), TiO_2 fabric (c) and BMF (d) (400× magnification).

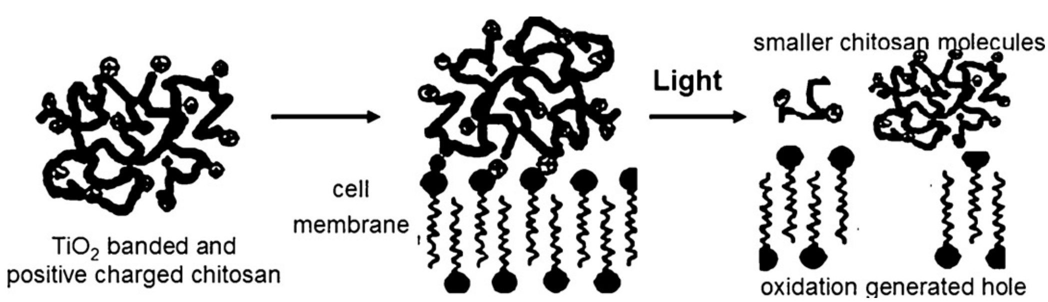

Fig. 11. Schematic of possible bactericidal mechanism of BMF.

and gradually cause the leakage of intracellular materials. Liu et al. [31] reported that $OH^{\bullet}$ and $O_2{}^-$ generated by TiO_2 photo-catalysis would attack the unsaturated bond of organisms.

The mechanism of the higher bactericidal activity of BMF under visible light can be attributed to the combined action of several factors as shown below: CS is a natural carbohydrate polymer with amino groups in its molecular structure. The chemical property of the amino group is rather active, and lone pair electrons of nitrogen atoms may attract H^+ and positive charges [8,9], or attach to the cell membrane through electrostatic force [10,14–16]. CS disturbs the outer membrane of the bacteria, while the simultaneous coupling of the strong photo-catalysis ability of TiO_2 further disrupts the barrier properties of outer membrane of the bacteria by the reactive oxygen species generated by CS/TiO_2 photo-catalysis under visible light. The perturbation of the outer membrane caused by both mechanisms above leads to the leakage of intracellular components. The opening up of the outer membrane moreover provides access for some substances including saline; CS/TiO_2 and free CS or TiO_2 particles, etc. Microorganism will finally be completely killed. The schematic of the possible bactericidal mechanism of BMF are shown in Fig. 11. Nevertheless, further studies are needed to elucidate the underlying mechanism of the bactericidal activity.

4. Conclusion

The TiO_2 photo-catalysis and bactericidal characteristics of chitosan (CS) were combined in a novel bactericidal and mildew-proof fabric (BMF) through immobilization of TiO_2–CS composite on cotton fibers, using cross-linking agent ECH at the optimum concentration of 2.5% (v/v) and applying a 30 min cross linking time. The bactericidal ratios of BMF for *E. coli*, *S. aureus* and *A. niger* reached 99.9%, 99.9% and 97.4% under visible light irradiation for 12 h, respectively. The study showed that the cell structure of microorganisms played an important role in the bactericidal activity of BMF. The long-lasting bactericidal ability of BMF was attributed to the *E. coli* cells being completely killed or broken instead of just being inhibited. The result was further supported by the micrograph pictures of the broken cell debris by BMF under visible light irradiation. The UV–vis transmission spectra revealed that BMF successfully outweighed the limitation of UV irradiation and had a strong absorption within the visible light spectral region. The results stress the potential of using BMF as an effective and promising bactericidal and mildew-proof material.

Acknowledgements

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