Antibacterial Capability of Triclosan Treated on Filter Fiber Materials

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Research Article

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Abstract

Antibacterial filtration material is an effectively control technique of airborne biological pollutant to purify indoor air. This study aims to assess the antibacterial capability of triclosan treated on three filter fiber materials: the glass fiber (GF), the non-woven fabric (NF) and the chemical fiber (CF). Triclosan was loaded on filtration materials by the impregnation method. E. coli, S. albus and S. aureus were used as test strains. It's found that the filter materials loaded with triclosan showed obvious antibacterial zone: the antibacterial zones against E. coli were 11.5 mm(GF), 13.2 mm(NF) and 11.0 mm(CF) respectively; zones against S. albus were 28.0 mm(GF), 21.0 mm(NF) and 25.0 mm(CF); zones against S. aureus were 21.5 mm(GF), 14.0 mm(NF) and 11.5 mm(CF). The percent reduction of bacteria of antibacterial fiber treated with triclosan against E. coli were 78.57% (CF) and 80.00% (GF), the percent reduction of bacteria of triclosan treated fiber against S. albus were 68.59% (NF) and 82.52% (CF), respectively. This research provided an effective antibacterial filter fiber material loaded with triclosan and it aids to reduce the transmission and harm of infectious diseases and to decontaminate the indoor environment.

1. Introduction

The controlling and terminating of airborne biological pollutants arouse people's attention in recent two years because of the epidemics of novel corona virus (COVID-19) in 2019 around the world. Therefore, an effective and environment-friendly control technique for bioaerosol is required to reduce the transmission and harm of infectious diseases and to decontaminate the indoor air effectively. According to the field literature, much effort has devoted to develop the active control technologies for airborne bacteria, including ultraviolet irradiation(Y. Yang et al. 2018), static electricity, microwave(Wu and Yao 2010), plasma(Bahri and Haghigat 2014), ozone, gas fumigation, lysozyme, photocatalysis (Lee 2016), air bactericide, etc. The above-mentioned active sterilization technologies have obvious sterilization effect and control effect on indoor microorganisms, but there are still some problems such as unstable sterilization effect (light blocking, electric field characteristics), secondary pollution (ozone or formaldehyde residue, heat pollution), high toxicity (chemical bactericide), poor application effect in the whole process of buildings and so on. Filters with antibacterial agent are effective in removing biological pollution in the air.

Recently, metal nanoparticles (silver (Abdulla et al. 2021)(Balagna et al. 2020), copper (Chowdhury et al. 2013)(Cao et al. 2014)) and natural plant essential oils (thyme essential oil (Salussoglia et al. 2020), Picea abies essential oil (Asanović et al. 2010) were widely used in antibiotics filter materials due to their sterilization efficiency, but their persistence of germicidal efficacy and high cost limited their use as a common antimicrobial agents. In addition, heavy metal ions also have certain biological toxicity to pose a threat to human health (Sánchez-López et al. 2020). Triclosan (2,4,4-trichloro-2-hydroxy-diphenyl ether, TCS) is well known as a broad-spectrum and environment-friendly antimicrobial and has been successfully used as an antibacterial agent in oral hygiene products (Kim et al. 2015), cosmetics (Halla et al. 2018) and the adhesive resin in dentistry (Machado et al. 2019).
Studies (Guo et al. 2020); (Aminu et al. 2019); (Paula et al. 2019); (Petersen 2014) on the application of triclosan in the field of stomatology found that triclosan has a good effect to disinfect the bacteria. And the more concentrated the triclosan was, the better antibacterial effect was observed. In a low concentration of antibacterial research, triclosan's antibacterial performance was manifested in inhibiting bacterial growth and reducing adherence to the polymer. Triclosan as an antimicrobial preparation of textile materials had also showed a good antibacterial effect (Karaszewska et al. 2017); (Cui et al. 2015); (Peila et al. 2013). At present, although filter materials with anti-microbe performance were ordinary, it still lacks the detailed information on the use and efficacy of triclosan as antibacterial agent applied to ventilation filter materials.

Therefore, the aims of this study are (1) to prepare three kinds of triclosan-loaded antibacterial filter materials and (2) to assess the antibacterial capability of triclosan treated on filter fiber materials. Chemical fiber (CF), glass fiber (GF) and non-woven fabrics (NF) were selected as the experimental filter materials, based on these materials widely used in ventilation filter. Triclosan was loaded on the filter materials by soaking method. In the study, *Escherichia coli* (*E. coli*, Gram-negative), *Staphylococcus albus* (*S. albus*, Gram-positive) and *Staphylococcus aureus* (*S. aureus*, Gram-positive) were chosen as the biological agents for testing the antibacterial capability of triclosan-treated filter materials.

2. Materials And Methods

2.1 Preparation of antibacterial fibers

As shown in Fig. 1, three kinds of filter materials used in this paper (glass fiber (GF), non-woven fabrics (NF) and chemical fiber (CF)) were treated as square-shaped of 12×12cm coupons. Half of the coupons were treated as soaking in alcohol triclosan solution. The consisted in the same certain concentration of alcohol solution.

Figure 2 shows the preparation of the triclosan-treated fiber coupons (Triclosan treated (TT)). First, add the triclosan to the beaker containing an alcoholic solution, until the white crystal appeared. After ten minutes for keeping, pour the supernatant of saturated solution of triclosan into the glassware, three filter materials were soaked in saturated solution of triclosan for five minutes. Then put the treated filter materials on petri dish (90 mm), and place into the dry oven (101-1AB) for 30 min at 50°C, then dry naturally for 24h in air. Subsequently, the secondary impregnation was carried out in the original supernatant, followed by the original drying process and then natural air drying. The other treatment is impregnating solution used as alcohol solution of the same concentration (Alcohol treated (AT)) and distilled water (No treatment (NT)).

2.2 Assays on antibacterial properties

2.2.1 Test organism and reagents
Antibacterial tests were performed with the bacteria of \textit{E. coli}, \textit{S. albus} and \textit{S. aureus}. Nutrient broth (Peptone 10.0g; Sodium chloride 5.0g; Beef paste powder 3.0g; Distilled water, 1000 ml; NB) is used to prepare the bacterial suspensions. AGAR medium (Peptone 10.0g; Sodium chloride 5.0g; Beef paste powder 3.0g; AGAR 15.0 g; Distilled water, 1000 ml; AM) is used in the culture of bacteria during the experiment, and 0.3 mol/l PBS buffer (Potassium dihydrogen phosphate 2.84g; Disodium hydrogen phosphate 1.36g; Distilled water, 1000 ml) is used to dilute the bacterial suspensions.

### 2.2.2 Preparation of bacterial suspension

For all the tests, the standard bacterial suspension was prepared starting from applying an inoculating loop to pick single colony up from the culture of the completed colony plate, placed in the NB and cultured in an oscillating incubator at 37℃ with rotating speed of 130r/min for 24h. Then, bacterial suspension concentration was measured by UV spectro-photometer at a wavelength of 660 nm. The concentration of the bacterial suspension was obtained using Eq. (1). The bacterial suspension was diluted in PBS to obtain a bacterial concentration of $10^8$ CFU/ml for the qualitative test and $10^6$ CFU/mL for the quantitative test.

$$C_{\text{sus}} = \text{OD}_{660\text{mm}} \times 10^9 \quad (1)$$

Where $C_{\text{sus}}$ is the suspension concentration, CFU/ml); OD$_{660\text{mm}}$ is absorbance value measured by UV spectrophotometer.

### 2.2.3 The qualitative antibacterial efficiency assays

Coupons preparation: two circles (diameter 24mm) were cut out of each coupon (ISO, 2004), then the coupons were exposed to UV light for 30 minutes to prevent contamination by bacteria.

The operation of the experiments was described as follows. Pour 10 ml AGAR medium (AM) into petri dish (90mm) as the underlying medium to solidify it. Then, 1 ml prepared bacterial suspension was transferred into 400 mL AM at 50℃ by pipette, shaking evenly and pouring 5ml of mixture into the petri dish with 10 ml AM. The coupons were placed in the center of the petri dish with tweezers. Press gently to ensure the contact between coupons and the medium, and send the petri dishes into incubator and cultured at 37℃ for 24h. Then, the size of the bacteriostatic zone was measured and recorded, and the average values of the two parallel samples were obtained, and also whether there was bacterial growth at the bottom of the filter material was observed. In addition, \textit{S. albus} and \textit{S. aureus} grow slowly in the experiments, resulting in a longer cultivation period in incubator (about 72 h), which caused the medium to crumple and rupture when adding less AM. Therefore, the amount of AM poured was increased to 20 ml in each disk appropriately in the course of operation.

### 2.2.4 The quantitative antibacterial efficiency assays

The antibacterial fiber was cut into the square shape coupons (5 mm×5 mm), which were weighed and put each 0.7g (ASTM, 2020; SAC, 2008), then irradiated under UV light for 30 minutes to sterilize.
Each of nine 250-ml flasks containing 70ml PBS buffer and 5 ml bacterial suspension, were divided equally into three groups. As shown in Fig. 3, one group was added distilled water, and the second group was added alcohol treated filter material, respectively, as blank and control group, which were put into incubator at 37°C with rotation speed of 250r/min, and shaken for 1min (named as “0” contact time). Then 0.6 ml bacterial suspension from each of the flasks was transferred into a 10-ml EP containing 5.4 ml of 0.03% PBS buffer solution, and diluted four gradients in turn. 1 ml bacterial diluent from each $10^2$ Eppendorf tubes was absorbed to make two parallel plates, then incubated in an incubator at 37 °C for 24 h-48 h and recorded the number of bacterial colonies in each plate.

As shown in Fig. 3, the last group of flasks was treated with triclosan filter material as the experimental group, three groups were put into an incubator at 37°C with rotation speed of 150 r/min, and the oscillation time was 18 h (named as “18h” contact time). Then dilute the bacterial suspension in each flask. And two parallel templates should be prepared for each dilution gradient, then placed all plates in the incubator, cultured at 37°C for 24 h-48 h and counted the number of colonies according to the diverse dilution gradient.

### 2.3 Evaluation methodology

#### 2.3.1 The qualitative antimicrobial evaluation

Qualitative antibacterial properties were evaluated according to the standard of ISO 20645 (ISO, 2004). With measuring the sterile area at the specimen edge, and the bacteriostatic zone was calculated using the Eq. (2):

$$H = \frac{D - d}{2} \quad (2)$$

where $H$ is the inhibition zone, mm; $D$ is the total diameter of specimen and inhibition zone, mm; $d$ is the diameter of specimen, mm.

After measuring the bacteriostatic zone, the coupon was removed from the medium and the bacterial reproduction in the contact area under the coupon was observed. Evaluate the antibacterial effect of the antibacterial treatment of the test specimen using Table 1 (ISO, 2004).
**Table 1**
Assessment of antibacterial effect

| Inhibition zone (mm) | Growth | Zone description              | under the coupons | Assessment     |
|----------------------|--------|-------------------------------|-------------------|---------------|
| > 1                  | none   | exceeding 1 mm                | no growth         | good effect   |
| 1–0                  | none   | up to 1 mm                    | no growth         |               |
| 0                    | none   | no inhibition zone            | no growth         |               |

### 2.2.4 The quantitative antibacterial evaluation

The antibacterial activity against *E. coli*, *S. albus* and *S. aureus* was evaluated with the quantitative method of assessing the antibacterial activity of textile materials (SAC, 2008). After oscillating contact for 18 h, the bacterial suspension was serially diluted and plated on the AM, and incubated at 37°C for 24 h- 48 h. Colonies that grew on the plate were enumerated. The bacteriostatic rate was calculated using the Eq. (3) (J. Yang et al. 2011):

\[
Y = \frac{W_t - Q_t}{W_t} \times 100\% \tag{3}
\]

Where \( Y \) is percent reduction of bacteria, %; \( W_t \) is the number of viable bacteria in the control sample after 18 h oscillation, CFU; \( Q_t \) the number of viable bacteria in the experimental sample after 18 h of oscillation, CFU.

### 3. Results And Discussion

#### 3.1 Performance of antibacterial filter material preparation

The antimicrobial filter materials obtained from the weight and thickness were detected and recorded. The changes in weight of the filter materials were shown in Table 2. Compared with those untreated, it can be seen evidently that the average weight gain of GF, NF and CF treated with triclosan were 2.77g, 2.25g, and 1.65g, respectively. It is explained that triclosan was attached to the filter material. The weight of the filter material treated with alcohol alone did not increased much. And the thickness of GF and NF with triclosan increased by 18.50% and 14.21% respectively, while CF decreased by 5.23%, compared with untreated filter materials. The explain of this phenomenon that the addition of triclosan might be led to the increasing in weight. The layered structure of CF was more loose and becomes compact with the treated of triclosan by soaking, so the thickness is reduced.
3.2 Results of antibacterial qualitative assays

3.2.1 The bacteriostatic properties of GF for *E. coli*, *S. albus* and *S. aureus*

As shown in Table 3, the bacteriostatic bands are all larger than 1 mm and there is no bacterial propagation at the contract area of coupons and AM. For GF treated with triclosan, the inhibition zone formed by *Escherichia coli* was the smallest (11.5±0.1 mm), while the bacteriostatic zone formed by *S. albus* and *S. aureus* were 28.0±0.1 mm and 21.5±0.1 mm, respectively, which is enough to demonstrate that GF treated with triclosan plays a good antibacterial effect based on Table 1. The bacteriostatic bands formed are shown in Fig. 4.

| Coupons | E. coli  | S. albus | S. aureus |
|---------|----------|----------|-----------|
| GF(TT)  | 11.5±0.1 | 28±0.1   | 21.5±0.1  |
| GF(AT)  | 4.5±0.1  | 10±0.1   | 15.5±0.1  |

“-” represents no bacterial growth at the contract area.

“+” represents bacterial growth at the contract area.

3.2.2 The bacteriostatic properties of NF for *E. coli*, *S. albus* and *S. aureus*

The sizes of bacteriostatic zones were recorded in Table 4. The inhibition band widths of NF treated with triclosan for *E. coli*, *S. albus* and *S. aureus* were 13.2±0.1 mm, 21.0±0.1 mm and 14.1±0.1 mm, respectively, which are all greater than 1 mm. There is no bacterial reproduction at the bottom of the coupons. Compared with the criteria in Table 1, the results showed that NF treated with triclosan has good antibacterial effect. However, the bacteriostatic zone of the alcohol-treated NF was smaller than that of
triclosan-treated, and there was *S. albus* growing at the bottom of the NF. The bacteriostatic zone of NF is shown in Fig. 5.

| Coupons | E. coli | S. albus | S. aureus |
|---------|---------|----------|-----------|
| NF(TT)  | 13.2±0.1 | -        | 21.0±0.1  |
| NF(AT)  | 4.7±0.1  | -        | 0.0±0.1   |

“-” represents no bacterial growth at the contract area.

“+” represents bacterial growth at the contract area.

### 3.2.3 The bacteriostatic properties of CF for *E. coli, S. albus* and *S. aureus*.

Table 5 shows the size of the bacteriostatic zone formed by CF after different treatments. The bacteriostatic zone of CF treated with triclosan was higher than the minimum limit of the bacteriostatic zone (1 mm) specified in the standard (ISO 20645 − 2004), which were 11.0±0.1 mm (*E. coli*), 25.0±0.1 mm (*S. albus*) and 11.5±0.1 mm (*S. aureus*), respectively, and no bacterial reproduction at the contract area of coupons and AM. The results showed that the CF with triclosan treated have good effect of bacteriostatic properties. The appearance of bacteriostatic zone is shown in Fig. 6. The results showed that there was no colony growth at the bottom of CF treated with triclosan, the antibacterial zone was obvious in CF treated with triclosan (Fig. 6).

| Coupons | E. coli | S. albus | S. aureus |
|---------|---------|----------|-----------|
| CF(TT)  | 11.0±0.1 | -        | 25±0.1    |
| CF(AT)  | 0.0±0.1  | -        | 3.0±0.1   |

“-” represents no bacterial growth at the contract area.

“+” represents bacterial growth at the contract area.

The appearance of bacteriostatic zone, to a certain extent, indicates that the added bacteriostatic agent (triclosan) had played a good antibacterial effect. Karaszewska et al.(2017) had also found that a wide range of bacteriostatic bands also appeared around the triclosan-loaded polylactide microparticles, based on the same evaluation criteria (ISO 20645 − 2004). Besides, it has a similar results that Cui et al. (2015)’s study of MgO-triclosan nanocomposite obtained obvious bacteriostatic zone by using a disk
diffusion assay. These field studies all demonstrate the reliability of triclosan's antimicrobial ability, and it is agreed with the qualitative experimental results in this study.

In addition, the physiological state of bacteria was a critical factor influencing the results of antibacterial testing. Experimental studies (Salussoglia et al. 2020) have shown that bacteria in the exponential phase exhibit larger logarithmic decrease than those in the stable phase. The bacteria used in the study belong to facultative anaerobe, so the growth of colonies is relatively slow and the amount of oxygen obtained by the colonies was relatively reduced after the colonies were mixed into the AM.

### 3.3 Results of antibacterial quantitative assays

In this study, there are six assays in the quantitative study, and 153 samples were carried in each quantitative assay. The percentage of the bacteria decrease in the quantitative experiments were shown in Table 6. The percent reduction of bacteria of GF and CF against *E. coli* were 80.00% and 78.57%. And the percent reduction of bacteria of NF and CF against *S. albus* were 68.59% and 82.52%, respectively, which can be considered to have antimicrobial effects. The antibacterial rate in this study is lower the reported results of Guo et al. (2020) (more than 99%) and Kamalipour et al. (2016) (more than 95% with 3 days). Paula et al. (2019) have reported that adding triclosan to resin composites can reduce the formation time of bacterial biofilms by more than 10 days and thus reduce bacterial adhesion. So, the concentration of triclosan and time of action, as well as the way of addition are one of the explanations for the different bactericidal effects mentioned above.

| Coupons | *E. coli* | *S. albus* |
|---------|-----------|------------|
| GF(TT)  | 80.00%    | --         |
| NF(TT)  | --        | 68.59%     |
| CF(TT)  | 78.57%    | 82.52%     |

“--” The number of bacteria (CFU) were out of the scope of standard

### 4. Conclusions

In the study, three kinds of triclosan-loaded antibacterial filter materials (chemical fiber, glass fiber and non-woven fabrics) were prepared and the antibacterial capability of triclosan treated on filter fiber materials were assessed by selected bacterial agent. In qualitative evaluation experiments, the obvious bacteriostatic zone indicated that the filtration materials with triclosan adhesion had good antimicrobial effect. The percent reduction of selected bacteria were more than 65% with three triclosan-loaded antibacterial filter materials. In the future, more work would be done on the application of the antibacterial filter materials in the air-condition system. This research provided an effective antibacterial filter fiber material loaded with triclosan and it aids to reduce the transmission and harm of infectious diseases and to decontaminate the indoor environment.
Declarations

Ethical Approval

Not applicable.

Consent to Participate

All authors have agreed to participate this study.

Consent to Publish

All authors have reviewed the final version of the manuscript and approve it for publication.

Author Contributions

Designed and conceived the experiments: Li Yanju. Conducted and performed the experiments: Li Yanju, MIAO Qinging, WANG Xinyu, HE Jinlu. Analyzed the data: Li Yanju, MIAO Qinging. Prepared and wrote the manuscript: Li Yanju, MIAO Qinging.

Competing Interests

The authors declare no competing interests.

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Availability of data and materials

The authors hereby certify that this paper consists of original, unpublished work which is not under consideration for publication elsewhere.

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Figure 1

the treated filter material ((a) No treatment (NT), (b) Alcohol treated (AT), and (c) Triclosan treated (TT); in each set of images, the left is GF, the middle is NF, and the right is CF.)
Figure 2

the preparation process of the filter materials treated by triclosan

Figure 3
the steps of oscillation and dilution during the quantitative experiments. ((a) blank group, (b) control group and (c) experimental group; Each group has three identical flasks and the operation procedure is exactly the same; “0” contact time: oscillated (a) and (b) for 1 minute at 24°C, 250r/min; “18h” contact time: oscillated (a), (b) and (c) for 18h at 24°C, 150r/min; The bacterial suspensions taken from each flask were successively diluted to five gradients (10^1, 10^2, 10^3, 10^4, 10^5))

![Figure 4](image)

**Figure 4**

Bacteriostatic zone of GF ((a) (d) E.coli, (b) (e) S.albus and (c) (f) S.aureus.; AT is on the top, TT is on the bottom.)
Figure 5

Bacteriostatic zone of NF ((a) (d) E.coli, (b) (e) S.albus and (c) (f) S.aureus.; AT is on the top, TT is on the bottom.)
Figure 6

Bacteriostatic zone of CF (((a) (d) E.coli, (b) (e) S.albus and (c) (f) S.aureus.; AT is on the top, and TT is on the bottom.)