

# Applying the Chlcone Antibacterial Filter for Indoor Bioaerosols Inactivating

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**Abstract.** This work considers the effects of using the flavonoids-chlcone pretreated filters (CPFs) as the antiseptic filters on the bioaerosol penetration. Two concentrations of chlcone solutions were used to coat on the filter. The *Escherichia coli* (*E. coli*), and *Bacillus subtilis* (*B. subtilis*) bioaerosols were generated using a collision nebulizer, as the challenged bioaerosols. The results show that culturable survival of *E. coli* bioaerosols through the untreated filter, the 0.01 wt% and 0.02wt% CPFs are around 78%, 66% and 55%, respectively. The results reveal that the culturable survival of *B. subtilis* bioaerosols through the untreated filter, 0.01 wt% and 0.02 wt% CPFs are approximately 80%, 72% and 67%. It suggested that the chalcone pretreatment did have an antiseptic effect on bacteria bioaerosols. The results showed that a higher concentration CPF corresponds to a lower bioaerosol survival. It also indicated that the chalcone could inactivate the bacteria when they were captured on the pretreated filters' surface. Moreover, the chalcone pretreated filters have a higher antiseptic on the *E. coli* bioaerosol than on the *B. subtilis* bioaerosol after those bioaerosols were captured.

## 1. Introduction

On average, most people spend as much as 87.2% of their time indoors where several indoor air pollutants are present[1]. Indoor bioaerosols represents an important issue because it causes respiratory and lung diseases in humans[2-5]. Accordingly, several air-cleaning technologies are employed to remove indoor bioaerosols[6-10].

Filtration is one of the most effective and reliable means of removing particulate matter and bioaerosols from a gas stream. However, microorganisms may grow on the face of filter and emit harmful volatile organic compounds or biotoxins [11-12](Maus et al., 2001; Moritz et al., 2001). For solving this problem, filter can be pretreated with antibacterial agent to inactivate indoor microorganisms[13-15]. Pyankov et al. (2010) combined tree oil and filters as the antibacterial filter to remove biological aerosols[13]. Huang et al. (2010) applied the *Melaleuca alternifolia* (tea tree oil) to inactivate fungal spores collected on fibrous filters[14]. Chen et al. (2016) indicated that filters coated with nanosilicate platelet supported silver nanohybrid could inactivate *Escherichia coli* and *Candida famata* effectively[15].



In recent years, the flavonoids has for several years been known to be antibacterial[16]. Chalcone, licorice's extract, is a kind of flavonoids. Many studies have demonstrated that chalcone has been used as an antibacterial agent. Ávila et al. (2008) applied the chalcones to test against bacterial strains, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Results indicated the tested chalcones were antibacterial against Gram-positive bacteria[17]. Nielsen et al. (2004) demonstrated that Licochalcone A has a significant antibacterial effect on Gram-positive bacteria. According to the relationship between structure and antibacterial ability, the phenolic hydroxyl groups of Licochalcone A are the major antibacterial components[18].

However, chalcone has seldom been employed in indoor environments to remove bioaerosols. Therefore, this work develops an antiseptic filter by pretreatment with chalcone. In this work, the survival of bioaerosol through the chalcone-pretreated filters (CPFs) was challenged with *E. coli* and *B. subtilis* bacteria bioaerosols to elucidate the effect of the sensitive and resistant strains of bacteria on survival through the chalcone-pretreated filters.

## 2. Experimental methods

**2.1 Chalcone Antibacterial Filter Media.** Polypropylene (PP) fibrous filters were employed in this study. PP filters were treated with chalcone. The original fiber diameter of the untreated filter was 20  $\mu\text{m}$ . Two concentrations (0.01 wt% and 0.02 wt%) of the Licochalcone A ( $\text{C}_{21}\text{H}_{22}\text{O}_4$ , molecular weight 338.40, purchased from Sigma-Aldrich Inc., Darmstadt, Germany) solutions were used for pretreating the tested filters. The Licochalcone A was dissolved in deionized water and filtered through 0.22  $\mu\text{m}$  pore size Millipore filter to remove residual as treating solution. The PP filters were soaked in the Licochalcone A treating solution for 1 minute and so became coated with the Licochalcone. After they had been soaked, the PP/ Licochalcone A filters were dried in an oven at 105°C for 12 hours.

**2.2 Tested Bioaerosols.** This work selected sensitive, resistant strains of bacteria (*E. coli* and *B. subtilis*) as the testing bioaerosols. In previous study, *E. coli* and *B. subtilis* was mostly evaluated for indoor-cleaning-technology germicidal test. The vegetative cells of *E. coli* (Bioresource Collection and Research Center in Taiwan, BCRC 10675) and endospores of *B. subtilis* (Culture Collection & Research Center in Taiwan, CCRC 12145) were selected as the model strains of bacteria. The *E. coli* was suspended in a phosphate buffer solution (pH 7.2), and the initial concentration was about  $10^5$  CFU/ml. The spores of *B. subtilis* were suspended in distilled water at a concentration of about  $10^5$  CFU/ml.

Three *E. coli* and three *B. subtilis* colonies from the agar plate culture to a conical flask containing 30mL tryptic soy broth (TSA, Difco Laboratories, Detroit) with a loop. Then, the TSA culture was incubated under a shaking condition of 85 rpm, for 16-24 h at 37°C. After the incubation, the TSA culture was centrifuged at 2500 rpm for 5 min. Then, we removed the resulting supernatant, added 30mL PBS solution (phosphate buffered saline, pH 7.2) and resuspended the *E. coli* and *B. subtilis* sediment. The PBS buffer solution was used to minimize the osmotic pressure between the microbial cellular fluids and the buffer solution. The above processes (except the incubation) were repeated twice to eliminate the TSA medium. The final PBS solution (*E. coli* stock) was used for the bioaerosol generation. The concentration of the viable *E. coli* and *B. subtilis* in the PBS solution was determined by counting colony-forming unit (CFU) on agar plates (serial dilution method).

**2.3 Experimental Set Up.** Figure 1 schematically depicts the experimental setup for the bioaerosol survival test of the CPFs. It comprises an aerosol generator, a neutralizer, a mixing column, a filter holder, a testing filter, an aerosol electrometer, relative humidity controlled system, an AGI-30 sampler (Model 7540 all glass impinger, ACE GLASS Inc., NJ, USA), and a flow meter.

**2.4 Bioaerosol Survival through Tested Filters.** The bioaerosol survival through a test filter is calculated from the following equation.

$$\text{Survival}_{\text{tf}} = C_{\text{upstream}} / C_{\text{downstream}}, \quad (1)$$

where is the bioaerosol survival ratio,  $C_{\text{upstream}}$  is the culturable concentration of bioaerosol upstream of the filter holder, and  $C_{\text{downstream}}$  is the culturable concentration of bioaerosol downstream of the filter holder.

**2.5 Bioaerosol Survival on the Tested Filters' Surface.** According to our pervious study[15], this work applied the following process to investigate the survival under the tested filters' surface. The microbial activity on filter over time was evaluated by comparing the survival ratio ( $S_f$ ). We defined that the survival ratio is the amount of culturable microorganisms on filter at a specific time to that at initial time, as calculated by Eq. (2)

$$\text{Survival}_{\text{ofs}} = N_{\text{e,t}} / N_{\text{e,o}}, \quad (2)$$

where  $N_{\text{e,o}}$  is the amount (CFU) of microorganism obtained from filter immediately by extraction process,  $N_{\text{e,t}}$  is the amount (CFU) of microorganism obtained from filter after  $t$  minutes by extraction process. To obtain the CFUs, the tested filters were immediately dismantled from holder and placed into a stainless steel chamber at RH 30% for 0 min and 10 min. The filters were then put into a 50 mL conical tube with 30 mL of extraction solution consisted of 0.1% peptone and 0.01% tween 80, and vortexed vigorously for 2 minutes followed by ultrasonic agitation for 10 minutes. The CFUs of the final extraction solutions were obtained by serial dilution and spread plate method. The experiment was conducted in duplicate.

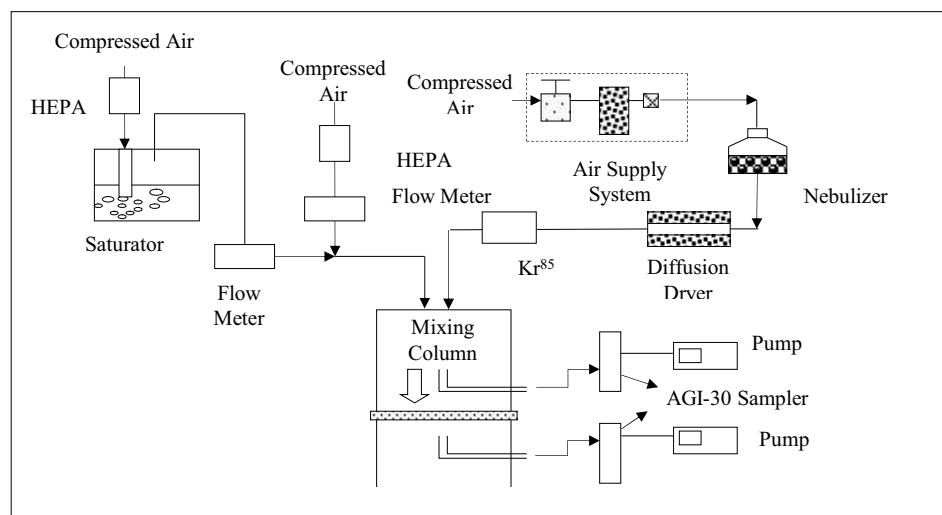


Figure 1. Schematic diagram of experimental set

### 3. Results and discussions

**3.1 Survival of Bacteria Bioaerosols through Chalcone Antibacterial Filter.** Figure 2 plots the survival of *E. coli* and *B. subtilis* bioaerosols through the untreated filter, 0.01 wt% and 0.02wt% CPFs (face velocity of 10 cm/s and RH of 30%) cultured by AGI-30 sampler. The results show that culturable survival of *E. coli* bioaerosols through the untreated filter, the 0.01 wt% and 0.02wt% CPFs are around 78%, 66% and 55%, respectively. The results reveal that the culturable survival of *B. subtilis* bioaerosols through the untreated filter, 0.01 wt% and 0.02 wt% CPFs are approximately 80%, 72% and 67%.

The pretreatment of Licochalcone A decreased the culturable survival of *E. coli* and *B. subtilis* bioaerosol. These findings reveal that the chalcone pretreatment has an antiseptic effect on bacteria

bioaerosol. It also revealed that the filter pretreated with a higher concentration of chalcone has a stronger antiseptic effect on bioaerosols.

The chalcone pretreated filters have a higher antiseptic on the *E. coli* bioaerosol than on the *B. subtilis* bioaerosol, mainly because *E. coli* is an environmentally sensitive bacterial strain and *B. subtilis* is a resistant bacterial strain. Therefore, *E. coli* bioaerosol is more easily removed by DPFs than is *B. subtilis* bioaerosol. In the previous investigations [9] indicated the *B. subtilis* was harder removal than *E. coli*. It is due to *B. subtilis* is the spore-type bioaerosol and *E. coli* is the cell-type bioaerosol. The tolerance of bacterial endospores is higher than that of the bacterial cell membrane. The multi-shell structure of spores could provide more protection than only cell membrane do.

**3.2 Bioaerosol Survival on the Chalcone Antibacterial Filters' Surface.** For understanding the survival ratio on the chalcone pretreated filters' surface, this work investigated the survival ratio on the different pretreated concentration CPFs' surface by challenged with *E. coli* and *B. subtilis* bioaerosol. The untreated filter was the control group and death of microorganisms on untreated filter was due to natural decay.

Experimental results showed that 0.01 and 0.02 wt% chalcone pretreated filters showed lower survival<sub>ofs</sub> (survival on the tested filters' surface) than that of untreated filter for both *E. coli* and *B. subtilis* bioaerosol. In other words, chalcone pretreated filters showed significant antibacterial effect.

The average survival<sub>ofs</sub> of *E. coli* and *B. subtilis* on untreated filter at 10 min were about 101% and 105%. It indicated that bacterial would not die on untreated filter. However, average survival<sub>ofs</sub> of *E. coli* for 0.01 and 0.02 wt% chalcone pretreated filters were 55% and 46%, at 10 minutes after *E. coli* bioaerosols were captured under relative humidity of 30%. Also, average survival<sub>ofs</sub> of *B. subtilis* for 0.01 and 0.02 wt% chalcone pretreated filters were 65% and 57%, at 10 minutes after *B. subtilis* bioaerosols were captured under relative humidity of 30%.

Comparison the survival<sub>ofs</sub> of the untreated filter and CPFs, the decrease is obviously. It indicated that the chalcone could inactivate the bacteria when they were captured on the pretreated filters' surface. Moreover, the chalcone pretreated filters have a higher antiseptic on the *E. coli* bioaerosol than on the *B. subtilis* bioaerosol after those bioaerosols were captured, mainly because *E. coli* is an environmentally sensitive bacterial strain and *B. subtilis* is a resistant bacterial strain. Therefore, chalcone pretreated filters demonstrated the antibacterial effect on *E. coli* and *B. subtilis* bioaerosols and the effect was significant.

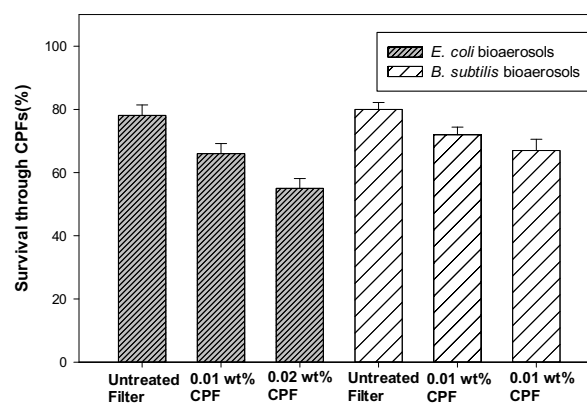


Figure 2. Survival through the untreated filters and CPFs for *E. coli* and *B. subtilis* bioaerosols.

#### 4. Summary

Experimental results demonstrated that the chalcone pretreatment has an antiseptic effect on *E. coli* and *B. subtilis* bioaerosols. The results showed that a higher concentration CPF corresponds to a lower bioaerosol survival<sub>tf</sub>. It also indicated that the chalcone could inactivate the bacteria when they were

captured on the pretreated filters' surface. Moreover, the chalcone pretreated filters have a higher antiseptic on the *E. coli* bioaerosol than on the *B. subtilis* bioaerosol after those bioaerosols were captured. This work might also offer a new indoor-controlling method for removal of bioaerosols.

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