Applying the Berberine-Pretreated Filter for Inactivating Bioaerosols

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Abstract: This work considers the effects of using the berberine pretreated filters (BPFs) as the antiseptic filters on the bioaerosol penetration. Two concentrations of berberine solutions were used to coat on the polypropylene fibrous filter. The *Escherichia coli* (*E. coli*), and *Bacillus subtilis* (*B. subtilis*) bioaerosols were generated using a Collison nebulizer, as the challenged bioaerosols. The effects of various factors, including the face velocity and the relative humidity on the bioaerosol collection characteristics were evaluated. Experimental results suggested the pretreatment of berberine did have an antiseptic effect on bacteria bioaerosol and increase the inactivation mechanism. The filter pretreated with a higher concentration of berberine has a stronger antiseptic effect on bioaerosols. The culturable survival of *E. coli* bioaerosols through the untreated filter, the 0.002 wt%, and 0.02 wt% BPFs are around 68%, 43% and 36%, respectively. In addition, the culturable survival of *B. subtilis* bioaerosols through the 0.002 wt%, and 0.02 wt% BPFs are around 66%, 51% and 43%, respectively. Moreover, the culturable survival of *E. coli* bioaerosol through the 0.002 wt% BPFs increased from 43% to 54% as the face velocity increased from 10 to 30 cm/s. These results indicated that the antiseptic of the BPFs decreased with face velocity.

Keywords: Berberine pretreated filters, Survival, Bacteria bioaerosols, Face velocity, Inactivating.

1. INTRODUCTION

On average, people spend as much as 87% of their time indoors [1]. Accordingly, indoor air quality is an increasingly important issue. Bioaerosols importantly affect indoor air quality because they cause various respiratory diseases [2-5]. Therefore, an increasing number of air-cleaning technologies are being adopted to remove indoor bioaerosols. Currently, bioaerosol-removal approaches include electret filtration [6, 7], electrostatic precipitation [8], ozone [9], ultraviolet germicidal irradiation [10], photocatalytic oxidation [11], negative ions [12, 13] plasma technology [14], electrolyzed water spraying [15] and oil coated antiseptic filter [16, 17].

Although many bioaerosol-controlling techniques are available, some procedures have very high removal efficiency toward bioaerosols while others have very low efficiency. This work proposes a new technique to remove indoor bacteria bioaerosols. In recent years, the berberine, coptis chinensis' extracts, was applied for antibacterial. The berberine is a quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids. Many studies have demonstrated that berberine has been used as an antiseptic material with, for example, antifungal activity [18] and antibacterial activity [19-21]. The main antiseptic mechanism of berberine is rapidly inhibiting the synthesis of ribonucleic acid (RNA) and protein of microorganisms while contact.

However, berberine has seldom been employed in indoor environments to remove bioaerosols. Therefore, this work develops an antiseptic filter by pretreatment with berberine. In this work, the survival of bioaerosol through the berberine-pretreated filters (BPFs) was challenged using two bacteria bioaerosols (*E. coli* and *B. subtilis*) to elucidate the effect of the sensitive and resistant strains of bacteria on aerosol penetration through the BPFs. The effects of the relative humidity and face velocity on the bioaerosol survival through the BPFs are also examined.

2. METHODS

2.1. Filter Media

Polypropylene (PP) fibrous filters were employed in this study. PP filters were treated with berberine. The weight of filters were measured by the electronic scale; the fiber diameter of the untreated and berberine pretreated filters was measured by the scanning electron micrograph (SEM) experiments; the filter thickness was measured by vernier caliper. The original fiber diameter of the untreated filter was 20 μm. Two concentrations (0.002 wt% and 0.02 wt%) of the berberine chloride solutions were used according to inhibition studies [22-24]. The berberine chloride ($C_{20}H_{18}ClNO_4$, molecular weight 317.81, purchased from Sigma Chemical Co., MO, USA) formulated as chloride salt that increasing solubility associated with

Table 1:   Characteristics of Tested Filters

Type	Material	Measured Weight of filter (g/m²)	Mean Fiber Equivalent Diameter[a] (μm)	Filter Thickness (cm)
Untreated	Polypropylene	60.5	25.2	0.11
0.002 wt % BPF	Polypropylene/ Berberine	67.3	25.3	0.12
0.02 wt % BPF	Polypropylene/ Berberine	71.2	25.3	0.12

[a]Diameter was measured by SEM experiments.

the original plant extract compounds. The berberine chloride was dissolved in deionized water and filtered through 0.22 μm pore size Millpore filter (Merck Millipore Co., Darmstadt, Germany) to remove residual as treating solution. The PP filters were soaked in the berberine chloride treating solution for 1 minute and so became coated with the berberine based on our previous experimental experience and demands (For understanding the additive antimicrobial effects of berberine in the study, the soaking processing have to increases the weight of PP filter without changing thickness and fibrous diameter significantly in case aerosol collection is affected). After they had been soaked, the PP/berberine filters were dried in an oven at 105°C for 12 hours. The characteristics of the untreated filter and BPFs were also presented in Table **1**.

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 [25, 26]. 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.

According to the previous research [27], the rod-shaped, gram negative *E. coli* represents a sensitive bacterial strain with a aerodynamic size of 0.63 μm and the rod-shaped, a aerodynamic size of 0.75 μm, gram-positive *B. subtilis* is regarded as very resistant for many adverse conditions. 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) [28].

2.3. Experimental Set Up

Figure **1** schematically depicts the experimental setup for the bioaerosol survival test of the BPFs. 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 i mpinger, ACE GLASS Inc., NJ, USA), and a flow meter.

The test aerosols, includes bioaerosols were aerosolized by using a Collison three-jet nebulizer (BGI Inc., Waltham, MA). The operating air pressure and flow rate of the nebulizer were 20 psig and 4.5ml/hr. The generated aerosols were dried by the diffusion dryer. The dried aerosols (bioaerosols and PSL aerosol) then passed through a Kr[85] radioactive source (model 3077, TSI Inc.), which neutralized them to the Boltzmann charge equilibrium. After it had passed through the neutralizer, the tested aerosol was delivered into the mixing column (the size of the mixing column was about 10 cm (L)× 10 cm(W) × 30 cm(H)), in which it was mixed with the diluted clean air. An aerosol electrometer (model 3068, TSI Inc, MN) was applied to detect the aerosol charge state in the mixing column. The face velocity through the tested filter was
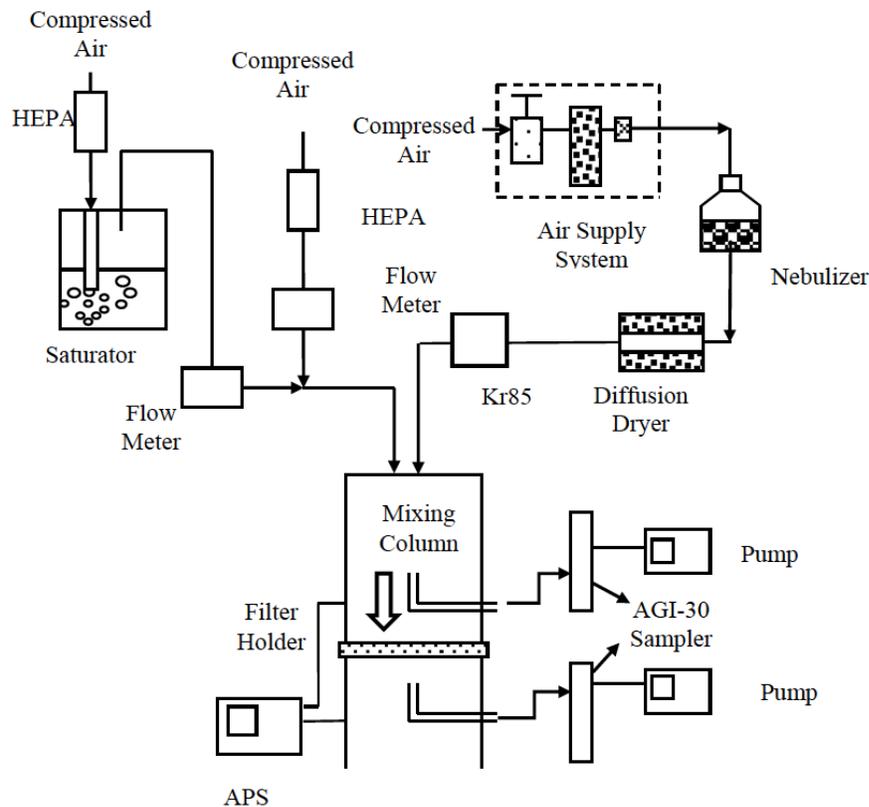
Figure 1: Schematic diagram of experimental setup.

controlled using a flow meter and a pump. The testing face velocities ranged from 10, 20, and 30 cm/sec and were applied to study the effects of the face velocity.
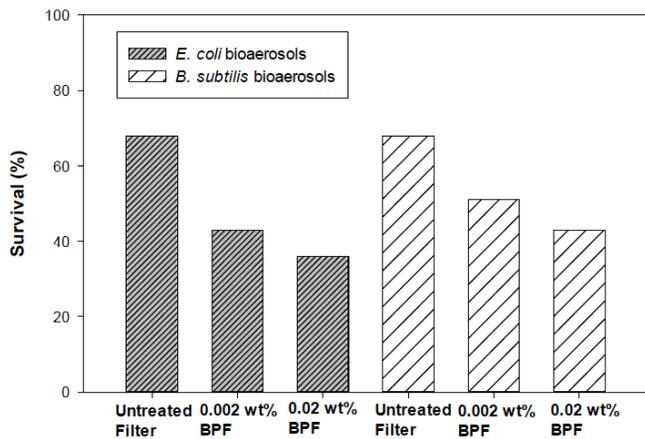
Figure 2: Survival through the untreated filters and BPFs for *E. coli* and *B. subtilis* bioaerosols.

This work considered the effect of relative humidity (RH) on the filtration characteristics. Three RHs were used in this work. The RH of the aerosol-flow stream was modified by changing the ratio of the flow rate of the dry gas stream to that of the humidified gas stream generated by the water vapor saturator. The final RH of the aerosol-flow stream was measured using a Q-Trak

Plus (Model 8552, TSI Inc.). Two relative humidity conditions for experiments were 30%, 50% and 70% that stood for dry, moderate, and humid condition respectively in this study. Tests were performed at least in triplicate for each type of filter, face velocity, RH and species of aerosol.

The bioaerosol survival through the test filters was determined as the ratio, $C_{upstream}/C_{downstream}$, where $C_{upstream}$ and $C_{downstream}$, were the culturable concentrations from the upstream and downstream of filter holder collected by an AGI-30 sampler. An AGI-30 impinger and a sampling pump (All Field Tech, Taiwan) were combined to be used for the bioaerosols sampling. This AGI-30 impinger was recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) and the International Aerobiology Symposium for sampling viable microorganisms [29]. The collection efficiency of the sampler is a function of the particle diameter and the sampling flow rate [30]. The sampling flow rate was 12.5 L/min and the sampling time was 6-8 min. The collected bioaerosol was recovered through serial dilution and cultured on TSA medium from after-sampling AGI-30 impinger. After 24 h culturing at 37°C, the viable colonies was calculated and converted to airborne concentration (CFU/m^3).

Moreover, the concentrations measured in the testing chamber by the AGI-30 sampler of the generated bioaerosol during each evaluated experiment were found to be stable within 1.0 hr. Each of these two bioaerosol concentrations were maintained at a range of 4 to 8 × 10^4 CFU/m^3. And the bioaerosol penetration through the testing filter were also sampling by an Aerodynamic Particle Sizer (APS, model 3320, TSI Inc, MN).

2.4. Bioaerosol survival Calculation

The bioaerosol survival through a test filter is given by

$$Survival = \frac{C_{downstream}}{C_{upstream}} , \qquad (1)$$

Where Survival is the bioaerosol survival ratio, $C_{uptream}$ is the culturable concentration of bioaerosol upstream of the filter holder, and $C_{downstream}$ is the culturable concentration of bioaerosol downstream of the filter holder.

3. RESULTS AND DISSCUSSIONS

3.1. Survival of Bacteria Bioaerosols through Berberine-Pretreated Filters

Figure **2** plots the survival of *E. coli* bioaerosols through the untreated filter, 0.002 wt% and 0.02wt% BPFs (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 0.002 wt% and 0.02wt% BPFs are around 66%, 48% and 39%, respectively. The pretreatment of berberine decreased the culturable survival of *E. coli* bioaerosol. Figure **3** shows the
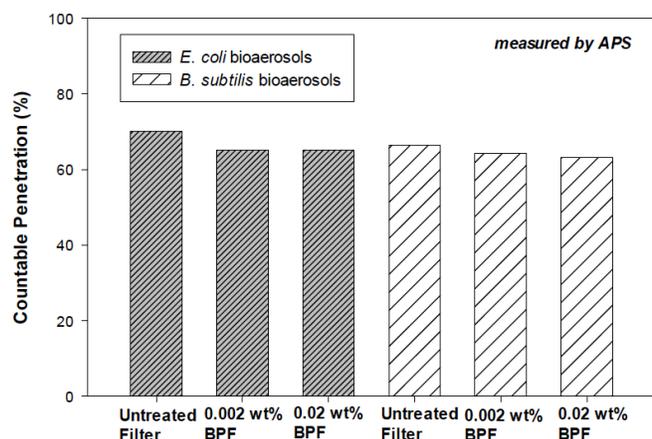
Figure 3: Countable penetration through the untreated filters and BPFs for *E. coli* and *B. subtilis* bioaerosols.

countable penetration of *E. coli* bioaerosols through untreated filter, 0.002 wt% and 0.02 wt% BPFs (face velocity of 10 cm/s and RH of 30%) sampled by APS are around 70%, 65% and 65%, respectively.

The pretreatment of berberine did slight affect the countable penetration of *E. coli* bioaerosol. A comparison between the results of culturable and countable penetration of *E. coli* bioaerosol through the BPFs indicates that the culturable survivals were lower than the countable penetrations obviously. These data were suggesting that the pretreatment of berberine only slight increase the mechanical bioaerosol removal mechanisms of the filters, but it raised the inactivation mechanism of the filters significantly.

Figure **2** also plots the culturable survival of *B. subtilis* bioaerosols through the untreated filter, 0.002 wt% and 0.02 wt% BPFs (face velocity of 10 cm/s and RH of 30%). The results reveal that the culturable survival of *B. subtilis* bioaerosols through the untreated filter, 0.002 wt% and 0.02 wt% BPFs are approximately 66%, 51% and 43%. The pretreatment of berberine decreased the culturable survival of *B. subtilis* bioaerosol. Figure **3** plots the countable penetration of *B. subtilis* bioaerosols through untreated filter, 0.002 wt% and 0.02 wt% BPFs (face velocity of 10 cm/s and RH of 30%) sampled by APS are around 66%, 64% and 63%, respectively. The pretreatment of berberine also slight affect the countable survival of *B. subtilis* bioaerosol. A comparison between the results of culturable survival and countable penetration of *B. subtilis* bioaerosol through the BPFs indicates that the culturable survivals were lower than the countable penetrations obviously. These data were suggesting that the pretreatment of berberine slight increase the mechanical bioaerosol removal mechanisms of the filters, but it raised the inactivation mechanism of the filters significantly. These findings, which were similar to those for *E. coli* bioaerosol, reveal that the berberine pretreatment has an antiseptic effect on bacteria bioaerosol.

The results indicate that the differences between the culturable and countable penetrations of *E. coli* bioaerosol through 0.002 wt% and 0.02 wt% BPFs are about 17% and 26%. The data also reveal that the differences between the culturable and countable penetrations of *B. subtilis* bioaerosol through 0.002 wt% and 0.02 wt% BPFs are about 13% and 20%. These findings demonstrate that a higher concentration BPF corresponds to a lower bioaerosol penetration and a larger difference between the culturable and

countable penetrations. It also revealed that the filter pretreated with a higher concentration of berberine has a stronger antiseptic effect on bioaerosols.

The pervious study [31] used the carbon nanotube filter to remove bioaerosols. Their results indicated that the removal efficiencies of CNT filters against *B. subtilis* aerosols were ranging from 10% to 95%. And for *P. fluorescens* bioaerosols, the efficiencies were in range of 5% to 60%. The BPF filter presented the removal efficiencies of *E. coli* bioaerosols through the 0.002 wt% and 0.02 wt% BPFs are around 57% and 64%. Thus, the BPF filter also demonstrated well inactiving ability on bioaerosols.

Further, the BPFs 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 BPFs than is *B. subtilis* bioaerosol. In the previous investigations [10, 32] indicated the *B. subtilis* was hardier 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. Effect of Face Velocity on Survival Rate through Berberine-Pretreated Filters

Figure **4A** plots culturable survival of *E. Coli* bioaerosol through the through 0.002 wt% and 0.02wt% BPFs at face velocities of 10, 20, and 30 cm/s (RH 30%). The experimental results show that the culturable survival of *E. coli* bioaerosol through the 0.002 wt% BPF increased from 43% to 54% as the face velocity increased from 10 to 30 cm/s. A paired t-test revealed a significant difference (p < 0.05) between survival of *E. coli* bioaerosol obtained with operating face velocity of 10, 20, and 30 cm/s suggesting that the survival decreased with an increasing face velocity. As displayed in Figure **4B**, the berberine-pretreated filter exhibits the same tendency with *B. subtilis* bioaerosol. The survival of *B. subtilis* bioaerosol through the 0.002 wt% BPF increased from 51% to 59% as the face velocity increased from 10 to 30 cm/s. Also, the paired t-test revealed a significant difference (p < 0.05) between penetration of *B. subtilis* bioaerosol obtained with operating face velocity of 10, 20, and 30 cm/s suggesting that the survival decreased with an increasing face velocity. Increasing the face

velocity reduces the residence time associated with bioaerosol attraction to the berberine on the surface of the BPFs. Therefore, the antiseptic of the BPFs falls as the face velocity increases.
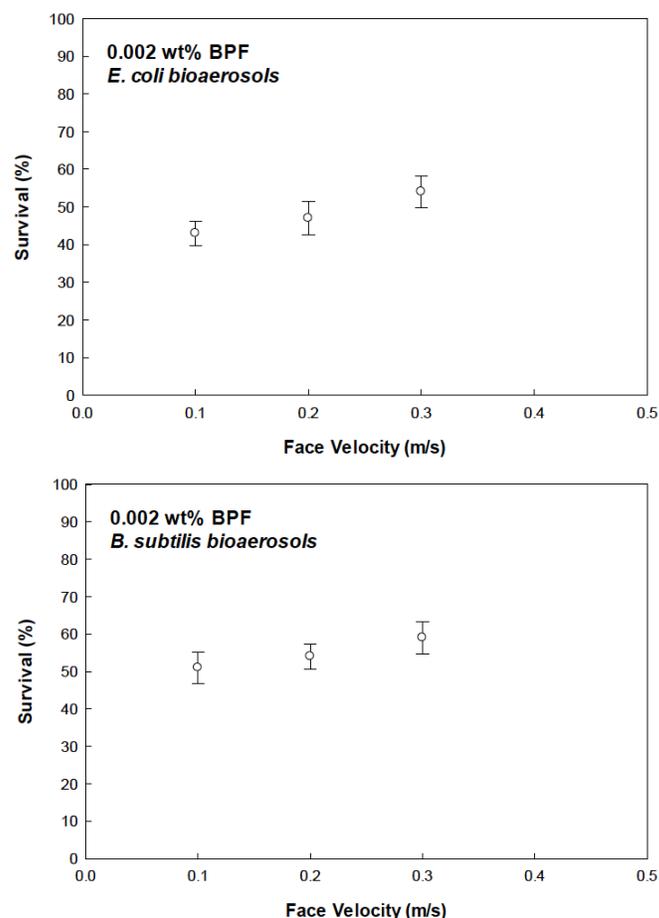
Figure 4: Survival through the untreated filters and BPFs for *E. coli and B. subtilis* bioaerosols at different face velocities.

3.3. Effect of Relative Humidity on Survival rate through the Berberine-Pretreated Filters

Three RHs (30%, 50% and 70%) were used herein to understand the effect of RH on bacteria bioaerosols. Figure **5** plots the *E. coli* bioaerosol survivals through 0.002 wt% and 0.02wt% BPFs at RHs of 30%, 50% and 70% (face velocity of 10 cm/s). The experimental results indicate that the survival of *E. coli* bioaerosols through BPFs was affected by RH insignificantly. For instance, the survival through 0.002 wt% BPF were 43%, 45%, and 45% at RH of 30%, 50%, and 70%. A paired t-test found no significant differences (p > 0.05) between penetrations obtained with different RH$_S$. Similarly, as presented in Figure **6**, the berberine-pretreated filter exhibited the same tendency with *B. subtilis* bioaerosol. The survivals of *B. subtilis* bioaerosol through the 0.002 wt% BPF were from 51%,

50%, and 51% at RH of 30%, 50%, and 70% (as shown in Figure **6**). A paired t-test also found no significant differences ($p > 0.05$) between survivals obtained with different RH_S. Thus, the experimental results demonstrated that RH had nearly no effect on the survival through the BPFs.
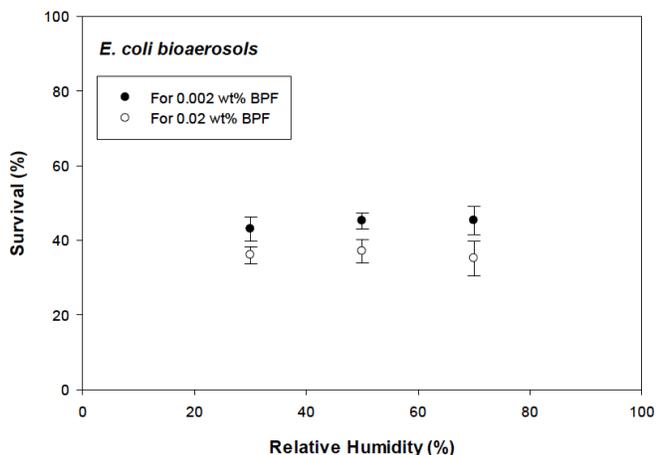
Figure 5: Survival through the untreated filters and BPFs for *E. coli* bioaerosols at different relative humidity.
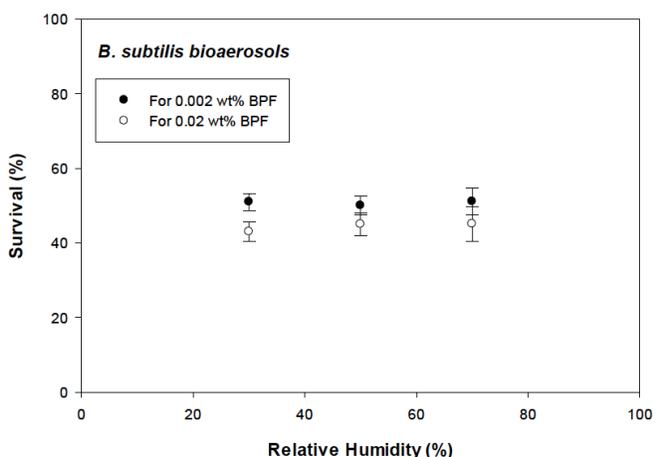
Figure 6: Survival through the untreated filters and BPFs for *B. subtilis* bioaerosols at different relative humidity.

4. CONCLUSIONS

Experimental findings revealed that the berberine pretreatment has an antiseptic effect on bacteria bioaerosol. The results demonstrate that a higher concentration BPF corresponds to a lower bioaerosol survival and a larger difference between the culturable survival and countable penetrations. It also revealed that the filter pretreated with a higher concentration of berberine has a stronger antiseptic effect on bioaerosols. Increasing the face velocity reduces the residence time associated with bioaerosol attraction to the berberine on the surface of the BPFs. Therefore,

the antiseptic of the BPFs falls as the face velocity increases. The experimental results also reveal that the survival of *E. coli* and *B. subtilis* bioaerosols through BPFs increase with RH. This work might also offer a new indoor-controlling method for removal of bioaerosols.

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