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The antibacterial performance of positively charged and chitosan dipped air filter media

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\textbf{ABSTRACT}

The bacteria and fungi captured on heat insulating materials or air filter media in the ventilation system in civil flights may reentrant into the indoor air which poses a threat to passengers’ health. The \textit{E. coli} and \textit{B. subtilis} bacteria survival rates on the heat insulating blankets typically used as padding materials inside the aircraft walls under the mimicked environmental condition were studied. Then the antibacterial performance of positively charged electret and chitosan modified filter media which can be used in the ventilation systems against \textit{E. coli} bacteria was investigated. Our results showed that the \textit{E. coli} survival rate deceased to less than 0.2% and that of the \textit{B. subtilis} decreased to less than 2.1% of the control samples on the hydrophilic and hydrophobic blankets under the mimick conditions after one flight cycle. The survival rate of \textit{E. coli} on the positively charged electret filter media decreased to less than 30% compared to the uncharged filter media after six hours due to the disruption of their metabolic balance by the positive charges on the fibers surface. Moreover, the \textit{E. coli} survival rate on the pure nylon, nylon/chitosan and chitosan dipped nylon-6 nanofibrous filter media decreased to 8.4%, 7.1% and 2.8% after 120 min, respectively. This study sheds light on fabrication of the eco-friendly antibacterial filter materials for the ventilation systems.

1. Introduction

During the last several decades, there have been increasing concerns within the scientific community over the effects of indoor air quality on health. The ventilation filters are applied in large quantities in buildings, automobiles and airplane cabins to protect human from particles, bacteria and virus in the indoor air. The influence of bacteria captured by filters on the human health has attracted a lot of attention of researchers. Voluminous of researches indicated that there were still considerable quantities of bacteria and fungi in the indoor air although the ventilation system was applied. The heat insulating blankets are used as padding materials inside the aircraft walls which may provide a dark habitat with condensed moisture for bacteria growth. The bacteria could be released and enter into aircraft cabin which threatens the passengers’ health.

Dechow et al. studied the contaminant in the cabin air of airbus aircrafts. Their result showed that the number of bacteria exceeded reported concentrations in other indoor environments \cite{1}. Liu et al. studied the culturable fungi/bacteria in the indoor air of 24 office buildings equipped with heating, ventilation and air conditioning (HVAC) systems in Beijing. Their results showed that the number of bacteria exceeded reported concentrations in other indoor environments \cite{1}. Liu et al. studied the culturable fungi/bacteria in the indoor air of 24 office buildings equipped with heating, ventilation and air conditioning (HVAC) systems in Beijing. Their results showed that the number of bacteria exceeded reported concentrations in other indoor environments \cite{1}. Liu et al. studied the culturable fungi/bacteria in the indoor air of 24 office buildings equipped with heating, ventilation and air conditioning (HVAC) systems in Beijing. Their results showed that the number of bacteria exceeded reported concentrations in other indoor environments \cite{1}.

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media such as ion and UV treatment and surface chemical modification are a variety of methods to endow the antibacterial property to filter. The filter surface can also be released into the indoor air [13].

The re-entrainment of the bacteria into the indoor air can cause high risks of respiratory diseases especially when the enclosed space has inadequate ventilation [6,7]. Moritz et al. studied the number of airborne bacteria and molds in front and behind the filters in a HVAC system. They concluded that during long periods of high relative humidity (>80% R. H.) a proliferation of bacteria on air filters with subsequent release into the filtered air occurred [8]. Many studies showed that, the air pollutants including the particles and bacteria in indoor air can be hazardous even at low concentrations especially for children [9–12]. Moreover, the volatile organic compounds produced by microbrial metabolism and the endotoxin emitted from the bacteria on the filter surface can also be released into the indoor air [13–15].

Given the threat posed by the bacteria accumulated on ventilation filters, the filter media with the antibacterial properties applied in the ventilation system can be a vital approach to reduce the concentration and potential risk of airborne and filter-associated bacteria [16]. There are a variety of methods to endow the antibacterial property to filter media such as ion and UV treatment and surface chemical modification [17–20]. In addition, a lot of studies focused on the antibacterial properties of the filters loaded with nanoparticles [21–26]. However, the UV treatment method is not convenient for the users and the release of ozone causes secondary pollution. Moreover, nanoparticles released from the material may have negative effects on human health [27–30]. Therefore, it is beneficial to find new ways to design the eco-friendly antibacterial filter media. As is well known, the majority of bacteria cell surface carries a net negative charge under most physiological conditions due to the proton dissociation of carboxyl, phosphate and amino groups [31–35]. The electret filter media with electrostatic charges added on fibers are widely used in the HVAC system because they can improve the particle collection efficiency without increasing the pressure drop [36–38]. The positively charged electret filter media used in the ventilation system may inactivate the bacteria due to the electrostatic interaction between fiber surface and bacteria without generating secondary pollution [39]. At the same time, chitosan, environmentally friendly and abundant in nature, can be used as bactericide since they can alter the cell permeability by the –NH₂ groups or bind with the DNA to inhibit the mRNA synthesis of the bacteria [40–42]. Therefore, chitosan can be used to modify the filter media for antibacterial application.

In this study, the survival rates of the Escherichia coli (E. coli) and Bacillus subtilis (B. subtilis) bacteria which are typically gram-positive and gram-negative bacteria in the lab controlled environmental condition of the aircraft cabin during flight was investigated in a climate chamber firstly. Then, the antibacterial performances of the positively charged electret filter media and chitosan modified filter media were studied. These methods provide possibilities for the design of antibacterial filter media in consideration of their environmental friendliness.

2. Materials and methods

2.1. Bacterial culture

Both E. coli and B. subtilis were used in the present work for survival rate experiments and antibacterial performance tests. Bacteria were cultured in LB broth medium in an incubator for 12 h at 37 °C, 50% RH in this study. All bacteria were washed with sterilized water once before usage in survival rate experiments. The bacteria concentration was measured by OD600 and colony formation unit (CFU) in LB agar plate for bacteria aerosol generation and bacteria survival rate testing, respectively.

2.2. The bacterial survival rate on the blankets in the lab mimicked civil flight cabin environment

Two kinds of hydrophilic and hydrophobic blankets (Fig. 1), which are deployed in civil flights typically, were used in this study to investigate the survival rate of E. coli and B. subtilis in different ambient environmental scenarios. The fibers in the hydrophilic blankets (HL) showed larger diameter and lower roughness compared with the fibers in the hydrophobic blankets (HB). Firstly, the actual temperature and the relative humidity in a civil flight where the two kinds of blankets were deployed were mimicked by a climate chamber (ICH110C, Memmert GmbH) in the laboratory. Based on the actual civil flight data, there were four phases in a cycle including parking, take off, cruise and landing process. The total duration of these four phases was 455 min. Five cycles were run totally in our study.

The schematic experimental setup of the bacterial survival rate on the blankets under the lab mimicked civil flight cabin condition is shown in Fig. 2. First, the two kinds of blankets were cut into pieces in the size of 2 cm × 2 cm and were sterilized by UV light for 2 h. Then 200 μL E. coli suspension (OD600 = 0.1, approximately 1.0 × 10⁶ cell/mL) was sprayed onto the UV treated blanket pieces. The 200 μL B. subtilis suspension (OD600 = 0.1, approximately 6.0 × 10⁷ cell/mL) was also loaded onto the UV treated blanket pieces in the same way. The blanket pieces loaded with bacteria were put into the incubator for 1, 2, 3, 4 and 5 cycles under the lab mimicked civil flight cabin condition.

Thereafter, the blanket pieces were put into the centrifuge tubes with 10 mL sterilized water after being taken out from the incubator. Then the tubes were shaken for 30 min by vortex to extract bacteria from the blankets to water. Then, 100 μL of E. coli or B. subtilis suspension was spread onto 90 mm LB agar plates. The agar plates were cultured in an incubator at 37 °C for 24 h in order to form colony for CFU counting.

For each of the experiments described here, data were collected from three replicate tests. For each test the bacteria eluate was plated in triplicate resulting in a total of nine data-points for each experiment and their average was used as the bacterial concentration. The survival rate of the bacteria was calculated as following

\[
SR = \frac{N_1}{N_0} \times 100\% \tag{1}
\]

where N₁ was the colony number of the survived bacteria on the blanket pieces after being cultured in the incubator for a certain number of cycles, N₀ was the number of the bacteria which were extracted from the loaded blanket pieces after the loading process immediately.

The decay rate of bacteria on filters was calculated based on an exponential decay model:

\[
Y = (Y_i - \text{Plateau}) \times e^{-Kt} + \text{Plateau} \tag{2}
\]

Here, Y is the survival rate of each kind of bacteria under the specific lab mimicked civil flight cabin environment, K is the decay rate presented as percentage per second. The half-life represents the time for half of the initial bacteria to die, which is calculated as ln(2) divided by K. The plateau means the quasi-constant bacteria survival rate after a long incubation period.

2.3. The antibacterial performance of the positively charged air filter media

To control the bacteria and potential risk, positively charged filter media were developed to inactivate bacteria in the ventilation system. The effect of the positively charged filter media on the E. coli bacterial survival rate was studied. The composite filter media that consisted of melt-blown fibers and PTFE membrane typically used for indoor ventilation systems were selected in this study. The morphology of the filter media was characterized by SEM and shown in Fig. 3. The PTFE membrane was the upstream layer and the melt-blown fibers layer was the
charged by the corona charging process. The schematic of the home-
with the diameter of 55 mm firstly. Then these pieces were positively
bacteria, the pieces were put into the climate chamber for incubation at
was the same as that in section 2.2. After being loaded with
measurement experimental setup was shown in Fig. 4 b. The measure
substrate of the Electrostatic Voltmeter (model 244A, TREK. Inc.,
were removed from the grounded plate of the charging device to the
substrate of the filter media. The filter media were cut into round pieces
of the filter piece [43, 44] and the voltage was 2 kV. The distances from
the needle tip to the metal mesh and from the metal mesh to the ground
metal plate were 2.5 and 0.5 cm, respectively. The charged filter media
were removed from the grounded plate of the charging device to the
substrate of the Electrostatic Voltmeter (model 244A, TREK. Inc.,
Lockport, NY., USA) for surface charge potential measurement. The
charged device was shown in Fig. 4 a. Before being charged, the
process of loading E. coli onto the positively charged filter media
was the same as that in section 2.2. After being loaded with E. coli
bacteria, the pieces were put into the climate chamber for incubation at
27 ± 2 °C, 95 ± 3% RH for 2, 4, or 6 h. The surface potential of the
filter media was measured by the Electrostatic Voltmeter at different
moments during the culture process to study the surface potential evolu-
After the incubation the filter media pieces were taken out and put
into centrifuge tubes with 10 mL distilled water. The bacteria were
washed off the filter pieces by shaking the tubes with vortex for 30 min
then 100 μL suspension was extracted from the tubes and spread onto
the LB agar plates. Thereafter, the loaded plates were cultured in the
incubator. The statistic method of using 9 data-point average to obtain
the bacterial survival rate was the same as that in section 2.2.

The bacterial survival rate on the charged filter media was calculated
as following

\[ SR = \frac{N'}{N} \times 100\% \]  (3)

where \( N' \) was the colony number of the E. coli bacteria on the positively
charged filter media after being incubated for a certain time. \( N \) was the
colonies number of the E. coli bacteria on the uncharged filter media
samples under the same test condition. The measurement on the un-
charged filter media provided the control experiment to account for
other factors which might lead to bacterial death, such as desiccation
stress, damage by handling, etc.

Bacteria were washed into sterilized PBS and stained with NucBea-
con Green and Propidium Iodide according to the suggestion of Via-
Quant™ Viability/Cytotoxicity kit for bacteria cells (Cat. A180,
GeneCopoeia, Inc.). The stained bacteria were imaged under a fluores-
cence microscopy (IX73, Olympus). In the fluorescence images, green
fluorescence indicated the live bacteria and red fluorescent indicated the
dead cells. The morphology of the E. coli bacteria on the positively
charged filter media after different time periods was characterized by
the environmental scanning electron microscope (ESEM, Quanta 450
PEG, FEI, USA), which could be used to observe the bacteria under high
relative humidity making smaller damage to the bacteria than the SEM.

2.4. Antibacterial performance of the chitosan treated nylon-6
nanofibrous air filters

To control the bacteria and potential risk, another method based on
chitosan treated electrospun nanofibrous filter media was investigated
for the antibacterial function. The nylon-6 nanofibrous filter media were
fabricated using similar methods as in Ju et al. [45]. Furthermore, the
chitosan component was added in this study. Briefly, nylon-6 pellets and
chitosan were dissolved in formic acid under stirring for 6 h for a
nylon/chitosan solution at 15 wt %. Nylon-6 was mixed with chitosan in
the weight ratio of 80/20. Nylon/chitosan nanofibrous filter media were
prepared by electrospinning with the 15 wt % nylon/chitosan solution,
whereas pure nylon nanofibrous filter media were prepared by elec-
trospinning with a 15 wt % nylon solution. A multi-jet electrospinning
system (NaBond Technologies Co., Ltd.), consisting of three spinnerets
and a rotating drum collector, was used for the fabrication of nano-
fibrous filter media. The distance between the spinneret array and the
drum collector was 10 cm. The voltage was set as 20 kV between the
spinnerets and the grounded collector. The extrusion rate was 0.5
ml.h⁻¹. Electrospinning for each filter media lasted for 2 h.

In another approach, the pure nylon nanofibrous filter media were
dipped in the chitosan solution (3 wt%) for 30 min after being fabri-
cated. The dipped filter media were dried in the oven for 24 h at the
temperature of 50 °C. The antibacterial experiment was the same as
mentioned above. The survival rate was calculated by equation (3).
Fig. 2. Schematic diagram of the experimental setup of the bacterial survival rate on the blankets in the lab mimicked civil flight cabin environment.

Fig. 3. The SEM image of (a) the top-layer surface and the (b) cross section of the air filter media used in this study.
3. Results and discussion

3.1. Bacterial survival rate on the blankets under lab controlled experiments to mimic civil flight environmental condition

Fig. 5 shows the lab controlled experiments to mimic civil flight environmental which was constructed in a climate chamber. The panels on the right are zoom-in views of the parts in the boxes on the left. The temperature and the relative humidity fluctuated during the parking and take off phases but were constant during the cruise phase. The temperature was constant while the RH increased during the landing phase. Furthermore, the lab experiment values in the climate chamber followed closely the set values according to the actual condition in the aircraft, which indicated that the climate chamber could mimic the real environmental condition in civil flight.
environmental condition in the civil flight very well.

The survival rate and the fluorescence microscope images of bacteria during the parking, take off, cruise and landing phases for one cycle under the lab mimicked civil flight cabin condition were shown in Fig. 6. The half-life and the K value which was the dead bacteria percentage per minute of these two types of bacteria were shown in Table 1. The survival rates of both E. coli and B. subtilis decreased quickly during the experiments. Even only after the parking phase, the survival rates of the two kinds of bacteria decreased to 3.13%–15.7%. This might be due to the dehydration of the bacteria at the beginning phase when the RH was mostly below 30% as it is well known that most of the bacteria can only survive for a substantial time when the humidity is above 80% [46–49]. Furthermore, the survival rates decreased more slowly during the takeoff and cruise phases and kept decreasing during the landing phase because of the nutrient loss. The fluorescence microscopy images in Fig. 6b and d are in agreement with the survival rate curves that the two kinds of bacteria died fast within 90 min.

In Fig. 6a, it can be seen that the E. coli on the hydrophobic blanket died faster than that on the hydrophilic blankets. Accordingly, Table 1 shows that the half-life of the E. coli on the hydrophilic blankets was significantly longer and the K value was much smaller than those on the hydrophobic blankets. This might be due to that the hydrophilic blankets could retain more moisture thus favorable for the survival of the E. coli. However, the B. subtilis on the hydrophilic blankets died faster than that on the hydrophobic blankets (Fig. 6c). It was also shown in Table 1 that the half-life of the B. subtilis on the hydrophilic blankets was significantly shorter and the K value was significantly larger than those on the hydrophobic blankets. Different decay rates of E. coli and B. subtilis on hydrophilic and hydrophobic blankets might be due to the different cell structures. E. coli is a typical gram-negative bacterium with sandwiched cell membrane including inner cytoplasmic cell membrane, cell peptidoglycan layer and bacterial outer membrane. In contrast, B. subtilis, a typical gram-positive bacterium, have thick peptidoglycan layer and inner cytoplasmic cell membrane [50]. After 455 min incubation, the survival rates of the E. coli and B. subtilis bacterial kept constant on the blankets in the climate chamber.

The half-life and K values of E. coli in hydrophilic blanket are significantly different from those values of E. coli in hydrophobic blanket by t-test (p < 0.01, n = 3). The half-life and K values of B. subtilis in hydrophilic blanket are significantly different from those values of E. coli in hydrophobic blanket by t-test (p < 0.01, n = 3).

The survival rates during five cycles were shown in Fig. 7. The survival rates of the bacteria decreased very quickly under the lab mimicked civil flight cabin environmental condition during the first cycle. This result was consistent with that in Fig. 6. Furthermore, the survival rate of the B. subtilis bacteria was higher than that of the E. coli bacterial under the same condition. The survival rate of B. subtilis still kept at 0.4% even after five cycles which was higher than that of the

| Table 1  |
| Decay rates of bacteria in the civil flight cabin environment. |
| Decay rates of bacteria | Half-life (min) | K ( × 10^-10%/sec) |
| | Mean | SD | 95% CI | Mean | SD | 95% CI |
| E. coli in hydrophilic blanket | 32.74 | 2.99 | (29.74, 35.72) | 3.53 | 0.15 | (3.23, 3.89) |
| E. coli in hydrophobic blanket | 17.58 | 0.74 | (16.82, 18.29) | 6.57 | 0.12 | (6.32, 6.87) |
| B. subtilis in hydrophilic blanket | 16.85 | 2.01 | (14.63, 18.65) | 6.86 | 0.36 | (6.19, 7.90) |
| B. subtilis in hydrophobic blanket | 22.53 | 1.41 | (21.06, 23.91) | 5.13 | 0.15 | (4.83, 5.48) |

Fig. 6. The survival rate curves and fluorescence microscope images (400 X) of E. coli (a and b) and B. subtilis (c and d) bacteria on the blankets cultured under the lab mimicked civil flight cabin environmental condition for one cycle (n = 3).
One main reason is that *B. subtilis* can produce endospores, which can remain viable for decades and are resistant to stressful environmental conditions such as drought, radiation, salinity, etc. Once the environment becomes suitable, the endospore will reactivate for reproducing [51].

This result indicated that the bacteria deposited on the indoor structures such as blankets, curtains, filters could survive for a long time which threatens our health since their spores can reproduce quickly in the right circumstances even if only a few spores survive. Given the reproduction characteristics of the bacteria, the antibacterial filters applied in the indoor ventilation system provide valuable benefit to protect human from the pathogenic bacteria.

### 3.2. Antibacterial performance of the positively charged air filters

Fig. 8a shows the surface potential decay vs. elapsed time on the positively charged filters after being loaded with *E. coli* bacteria. The average initial potential of the charged filter media was 485.9 V. The results showed that the surface potential of the loaded filter media decreased more quickly than that of the unloaded filter media. This may be because that the positive charges on the surface of the filter media were neutralized by the negatively charged bacterial. At the same time, the water molecules of the bacterial suspension may accelerate the loss of the charges [52,53].

The survival rate of the *E. coli* bacteria loaded onto the surface of the positively charged air filter media after being cultured in the incubator for different durations was shown in Fig. 8b. Compared to the control samples, the *E. coli* bacterial survival rate decreased to 62.4% after 2 h and 17.8% after six hours. This result indicated that the positively charged filter media inhibited the growth of bacteria. This might be caused by that the positive charges on the fiber surface could interact with the negative charges in the outer envelope of the bacteria leading to the loss of replication ability of bacteria DNA and the protein inactivation [54,55]. Moreover, the survival rate decreased as the incubation time increased due to the longer contact time between the bacteria and the positively charged filter media. Furthermore, the survival rate was much higher compared to those in the blanket experiments. The reason was that the survival rate here was compared to the control sample (uncharged filter) rather than the initial number.

### 3.3. The morphology of the *E. coli* bacteria on the positively charged filters

The ESEM images (Fig. 9) reveal the morphological evolution of the *E. coli* loaded onto the positively charged filter media. It can be seen that the *E. coli* bacteria were plump at the beginning and became increasingly flatter after contact with the positively charged filter media. The edge of the bacteria could be discerned after 360 min. This revealed that the interaction between the positive charges on the fiber surface and the negatively charged bacteria could change the bacterial morphology. The schematic of the interaction between the bacteria and fibers was shown in Fig. 10 and the interaction would disrupt the metabolic balance of the *E. coli* bacteria [55]. Therefore, more *E. coli* bacteria died on the positively charged filter media than on the uncharged filter media.
3.4. The survival rate of the E. coli bacteria on the chitosan treated air filters

The survival rate of the E. coli bacteria on various nanofibrous filter media was shown in Fig. 11. It can be seen that the E. coli bacteria survival rate decreased sharply after 120 min even on the pristine nylon-6 nanofibrous filter media. Moreover, it was the lowest on the chitosan dipped nylon-6 nanofibrous filter media and decreased to zero after 240 min. This result indicated that the chitosan on the surface of the filter can inhibit the bacteria effectively. The effect may arise from the interaction between the positively charged amino groups and the bacteria which could disrupt the synthesis and dissolution balance of the cell wall of the bacteria, resulting in leakage of intracellular constituents and bacteria death [41,42,56,57]. Furthermore, the bacterial survival rate on the nylon/chitosan blend electrospun nanofibrous filter media was also lower than that on the pristine nylon-6 media but higher than that on the chitosan dipped nylon media. This difference may be due to the interaction between nylon-6 and chitosan molecules. The hydrogen bonds could form between the –C=O groups in nylon-6 and the –NH₂ groups in chitosan, which may reduce the amount of the –NH₃⁺ groups formed with the water molecules [58]. Due to the homogeneous blend in electrospun nanofibrous filter media, there were less chitosan molecules on the fiber surface and less chitosan-bacteria interaction than that were dipped on nylon-6 nanofiber. Thus, the antibacterial ability of the nylon-6/chitosan blend electrospun nanofibrous filter media was lower than that of the dipped nylon-6 nanofibrous filter media.

4. Conclusions

In this work, we measured the decay rates of both gram-positive and gram-negative bacteria under different environmental scenarios and developed two antibacterial methods based on positively charged and chitosan dipped air filter media to control the bacteria and potential risk. The following conclusions can be made: (1) Model bacteria decayed...
quickly in the civil flight conditions but still a few might survive after one flight cycle; (2) Both positive charges and chitosan on filter media showed effective antibacterial capacity.

These basic decay rates are important parameters to study the properties of airborne microorganisms, such as the life span and morphology. The decay rates can also be used in the bioaerosol research for the life span and morphology of airborne microorganisms, such as the life span and...

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