Recovery of Bacteria in Filtering Facepiece Respirators and Effects of Artificial Saliva/Perspiration on Bacterial Survival and Performance of Respirators

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ABSTRACT

This study seeks the optimal method for recovering loaded bacteria from filtering facepiece respirators (FFRs) and investigates the effects of artificial saliva (AS), artificial perspiration (AP) and storage conditions on the survival of bioaerosols and the filter performance of FFRs. Bioaerosols were generated using a Collison nebulizer in a test system and loaded on either N95 or surgical masks. Elution using centrifuge at 3500 rpm for ten min followed by vortexing for one min yielded a high relative survival (RS) rate of airborne *Bacillus subtilis* (BS) spores. When AS was added to the N95 FFR, the RS of BS declined during the first eight hours of storage and then increased to reach its highest value after 24 hr of storage. The worst case with the highest RS was at 37°C and 95% RH ($p < 0.001$). When AP was added to the N95 FFR and stored under the worst conditions, RS increased by over 100% during eight hours of storage. When AS was added to a surgical mask, the RS also increased by over 100% in eight hours of storage, but when AP was added to the surgical mask, RS immediately declined. When *Escherichia coli* (EC) were tested, their RS was lower than those of the BS samples. (Following loading with bacteria, the particle penetration and filter quality factor (q_f) increased ($p < 0.001$) but the slope of the linear regression between the pressure drop (Δp) and the flow rate through the filter was not statistically significantly changed ($p = 0.233$). In conclusion, AS and AP increased the survival of BS; AP was especially effective in N95 masks and AS was especially effective in surgical masks.

Keywords: Respirator; Bioaerosols; Filtration; Personnel protection; Respiratory health.

INTRODUCTION

Disposable filtering facepiece respirators (FFRs) are frequently utilized to prevent the transmission of inhalable particulates and infectious or allergenic bioaerosols into the respiratory tract. During an outbreak, the timely distribution of appropriate protective equipment to workers is recommended, especially when other actions to control respiratory exposure are unfeasible. Similar conditions are observed upon various emergent disasters. Unfortunately, deficiencies or delays in the distribution of FFRs sometimes occur and FFR reuse is required (Fisher *et al.*, 2014).

One concern associated with the reuse or prolonged usage of FFR is the increased risk of potential microorganism growth on the FFR filter owing to improper handling, storage or reuse (Wang *et al.*, 1999). Bacteria that survive on the FFR promote reaerosolization through inhalation since they reproduce in the filter matrix, eventually detaching from the fibers and entering the human respiratory system via flowing air through the filter (Wang *et al.*, 1999). In particular, when a mask is used repeatedly, the reentrainment of bacteria may affect its wearer's health.

Few investigations have focused on the survival of bacteria that are captured by a disposable FFR (Pasanen *et al.*, 1993; Brosseau *et al.*, 1997a). Wang *et al.* (1999) found that robust *Bacillus subtilis* (BS) spores remained viable on an FFR for more than 13 days of testing, and observed the penetration of surviving microorganisms into the FFR. Previous studies have not considered general storage conditions (25°C and 60% RH), the present investigation does so.

Some researchers have investigated the multiplication of bacteria that are loaded onto heating, ventilating and air

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conditioning (HVAC) filter media. Under static and dynamic conditions, no multiplication of *B. subtilis* occurs owing to a lack of available nutrients (Maus *et al.*, 2001). However, the effects of such nutrients as saliva and perspiration on the survival of bacteria must be evaluated further.

In this study, the effects of liquid nutrients, including artificial saliva and perspiration, on the survival of loaded bacteria are experimentally examined. The survival conditions for bacteria on a used FFR, with respect to contact with human secretions at various temperatures and humidity levels and settling times, are investigated. To the best of the authors' knowledge, this study is the first systemic investigation of the effects of artificial liquid nutrition on the survival of collected bioaerosols on FFRs and the recovery thereof.

MATERIALS AND METHOD

Bioaerosol Generation and Sampling System

This is mainly concerned with the relative survival (RS) of airborne bacteria that are loaded onto masks using various elution methods, nutrients, storage temperatures and relative humidity (RH). A test system is established in which a Collison nebulizer generates bacterial particles in a chamber with four filter holders with a diameter of 45 mm (Fig. 1). Each holder was connected to a suction pump. Following aerosolization, the pumps were turned on and bioaerosols were then loaded onto the test filters, simulating
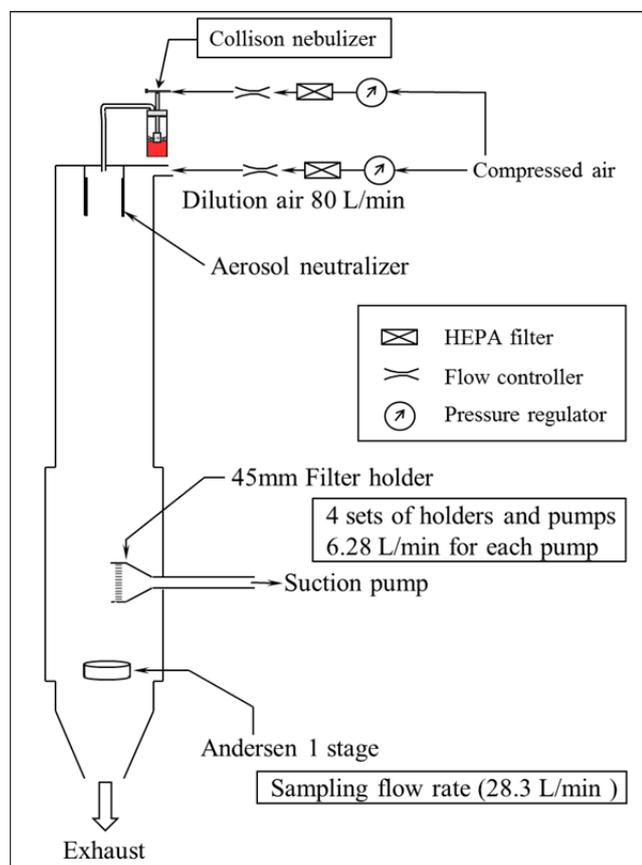

Fig. 1. Test system.

the respiration of heavy-duty workers for 30 min.

Bacteria: *Bacillus subtilis* (BS) (CCRC 12145) and *Escherichia coli* (EC) (ATCC 25922) were utilized representing hardy endospores and sensitive cells, respectively (Li *et al.*, 1999). BS is a Gram-positive bacterium and was used herein to create an endospore suspension. These spores can resist severe environmental burdens and are frequently used in experiments (Li *et al.*, 1999).
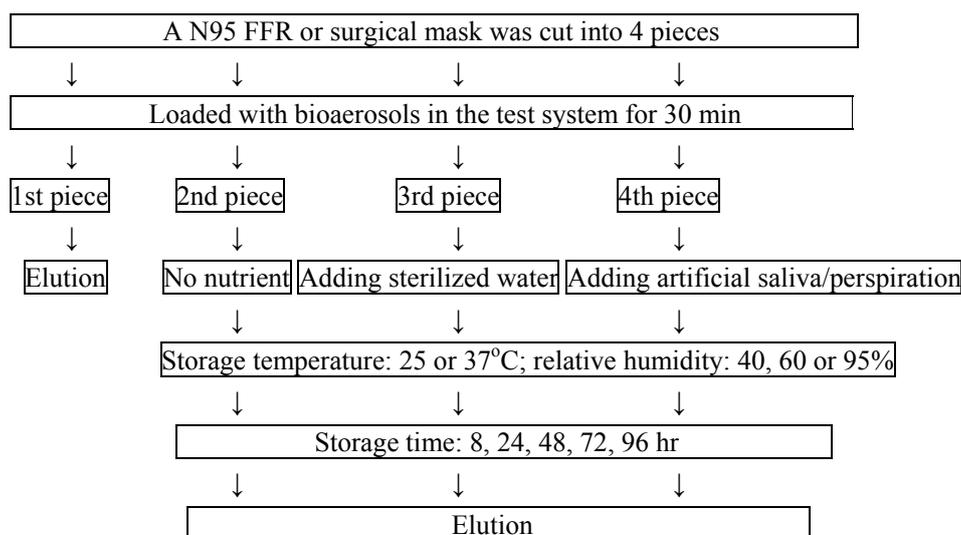
Bioaerosol generation: A Collison nebulizer (refluxing six-jet modified MRE-type short-form, Model NSF CN-31/1) was used to generate biological aerosols following Li *et al.* (1999). After incubation and treatment, 55 mL of BS endospores or the EC suspension was placed in the Collison nebulizer at a pressure of 25 psi to generate a stable concentration of bioaerosols for at least two hours, which was longer than the period of bacterial loading (30 min) in each test. The rate of flow of the dilution air was 80 L min^{−1}, as required for the vacuum pumps downstream. The medium (Trypticase soy agar, TSA) was placed in an Andersen 1 stage sampler (Andersen Inc., Atlanta, GA) to ensure the cultivability and stability of the generated bioaerosols in the system, and the locations of the holders and the Andersen sampler were carefully set to avoid mutual interference .

Filters: The N95 mask (Dust Respirator 8210; 3M, St. Paul, MN) was utilized as the test filter in this experiment because of its popularity in Taiwan during the SARS outbreak in 2003 (Kao *et al.*, 2004). A pleated surgical mask with ear loops was tested for comparison (Chen *et al.*, 1994; McCullough *et al.*, 1997). The filter material was polypropylene (PP) and the exterior of the mask was a mixture of PP and polyester. To simulate the breathing flow rate of heavy-duty workers (85 L min^{−1}) (Brosseau *et al.*, 1997b), the calculated surface velocity was 8.3 cm s^{−1} for the whole mask- close to that in an earlier study (Wang *et al.*, 1999). In each batch test, four pieces with a diameter of 45 mm were cut from a mask to fit the filter holder; the effective diameter was 40 mm, similar to that used by Wang *et al.* (1999); the filtration area was calculated as 12.56 cm[2]. Considering the same surface velocity, the flow rate of the vacuum pump that was connected to each filter holder was set to 6.28 L min^{−1}, which was maintained using a mass flow controller. The sampling time was 30 min, determined by trial and error to prevent overloading by bacteria that collected on the filters in the test chamber.

Type of Nutrients added to the Bacteria-Laden Filters

After loading with bioaerosol, the first piece was immediately eluted to serve as the reference (Fig. 2). No nutrient was added to the second. To simulate contamination by human sweat and saliva during the wearing of a mask, sterilized water and nutrient (artificial saliva or artificial perspiration) were added to the center of the inside of other two pieces using pipettes, respectively. These last three pieces were placed in separate petri dishes using sterilized forceps and then stored in an incubator at a constant temperature and humidity (Model: HONG-YU, HM-80, Taichung, Taiwan). The variation of the bacterial load on each filter was pretested and found to be negligible.

Artificial saliva (AS): Distilled water was blended with

A N95 FFR or surgical mask was cut into 4 pieces

↓ ↓ ↓ ↓

Loaded with bioaerosols in the test system for 30 min

↓ ↓ ↓ ↓

1st piece	2nd piece	3rd piece	4th piece

↓ ↓ ↓ ↓

Elution	No nutrient	Adding sterilized water	Adding artificial saliva/perspiration

↓ ↓ ↓

Storage temperature: 25 or 37°C; relative humidity: 40, 60 or 95%

↓ ↓ ↓

Storage time: 8, 24, 48, 72, 96 hr

↓ ↓ ↓

Elution

Fig. 2. Sampling flow chart and test conditions.

the constituents that are presented in Table 1 (Sali lube, Sinphar Pharmaceutical Co. Ltd., Taipei, Taiwan).

Artificial perspiration (AP): The artificial perspiration comprised the constituents that are presented in Table 2 (JIS L 0848 Japanese standard) (Vig *et al.*, 2007).

Both AS and AP were autoclaved before use (Wang *et al.*, 1999).

Determining Optimal Volume of Nutrients

To ensure that the survival rate of the challenge bioaerosols would not be underestimated, the optimal conditions for recovering the loaded bacteria from the FFR were determined. Two major physical factors were considered; they were the maximum volume of liquid nutrition that was added to the filters and the elution method.

The selected volumes of AS on the N95 mask were 0.5, 1.0, 1.3, 1.5, 1.7, 2.0, 2.5 and 3.0 mL. When the surgical mask was used as the challenge filter, 0.1, 0.3, 0.5, 0.7, 1.0 and 1.5 mL of AS were added. The liquid wicked and spread homogeneously on the filter. In this investigation, at least

Table 1. Constituents of artificial saliva[a].

Constituent[b]	Quantity, mg L^{-1}
Sodium chloride	0.844
Potassium chloride	1.200
Anhydrous calcium chloride	0.146
Magnesium chloride 6H$_2$O	0.052
Dibasic potassium phosphate	0.342
Sorbitol solution 70%	60.000
Hydroxyethyl cellulose	3.500

[a] Artificial saliva was prepared by combining the listed constituents to achieve a pH of 6.3.
[b] To model the worst case that most favors bacterial survival, methyl paraben, a germicide, was not added to the artificial saliva, enough though actual saliva contains antimicrobial proteins. The bacteria that are present in the mouth and the antimicrobial effectiveness of saliva depend on the health of the mask wearer.

Table 2. Composition of artificial perspiration.

Component[a,b]	g L^{-1}
Sodium chloride, NaCl	5
Sodium D-pantothenate	5
Disodium hydrogen phosphate, Na$_2$HPO$_4$·12H$_2$O	5
Glucose (anhydrous)	5
Lactic acid (85%)	5
DL-aspartic acid	0.5
Histidine	0.5

[a] Consistent with JIS L 0848 Japanese standard.
[b] Acetic acid was added to adjust pH to 3.5.

three trials were conducted in each set to obtain average values and standard deviations.

Although the optimal values of all parameters had not yet been determined, BS spores were utilized as the challenge bioaerosols. Sterilized forceps were used to clip each filter into a petri dish, which was then placed for 24 h in an incubator that was set to 37°C and 95% RH. The bacteria were then eluted using a 3500 rpm centrifuge (Model: SEING-DIA, CN-3600, Taichung, Taiwan) for 10 min. During the elution procedure, the test filter was placed in a 50 mL centrifuge tube and then 20 mL of sterilized water was added to soak the filters completely. Following elution, the tube was vortexed for one min to ensure thorough mixing of the solution and triplicate 0.1-mL samples were smeared onto TSA media. The colonies were enumerated following incubation at 37°C for 48 h.

The relative survival (RS) was calculated as follows (Lin and Li, 1998, 2003).

$$Relative\ Survival,\ RS = \frac{CFU_T}{CFU_0} \times 100\% \qquad (1)$$

where CFU$_T$ = number of colony-forming units that were eluted from the filter following storage,
CFU$_0$ = number of colony-forming units that were eluted from the filter before storage.

Optimal Method for Eluting the Loaded Bacteria from the Filter

Numerous methods exist for eluting loaded bacterial particles from a test filter. Four commonly available instruments were utilized to compare the numbers of recovered colonies and to determine the optimal elution method. The first three instruments were an orbital water shaker (Model: Yite, TS-520, 200 rpm, 3.3 Hz, Taichung, Taiwan), an ultrasonic oscillator (Model: Blossom, LEO-3002, oscillating frequency: 46 kHz, Taipei, Taiwan), a vortex touch mixer (Model: Digisystem, VM-2000, 3000 rpm, 50 Hz, 100 mm of vortex head, Taipei, Taiwan), which were utilized by Wang *et al.* (1999), and the fourth was a centrifuge, which was mentioned in the preceding paragraph. The elution time was set to 10 min in all methods. Before elution, the test filter was loaded with airborne BS spores and an optimal volume of sterilized water or AS (as determined from earlier trials); the loaded filters without added nutrition were the controls. All of the samples were stored at 37°C and 95% RH for 24 h.

The optimal centrifugal speed was determined before the elution methods were compared. Various speeds were set; they were 1500, 2500, 3000, 3500, 3700, 4000 and 4500 rpm, which are equivalent of 352 g, 978 g, 1409 g, 1917 g, 2143 g, 2504g and 3170 g, respectively.

Eq. (1) was used to determine the highest number of recovered colonies (as RS) in the centrifugal speed test and the optimal speed was thus determined and used in all centrifugal tests.

Storage Conditions after Bacteria and Nutrients were Loaded onto Test Filter

After the optimal volumes of nutrients and the optimal elution method were determined, the effects of the storage conditions on the RS of two bacterial strains were evaluated. The storage conditions included duration of storage, temperature and RH (Fig. 2). Each parameter was set to at least two values for comparison; three RHs (40, 60 and 95%), two temperatures (25°C, room-temperature, and 37°C, body temperature), and five storage periods (8, 24, 48, 72 and 96 h) were considered.

The lower two RH values are the lower and upper limits on the RH in a general air-conditioned indoor environment. The RH of 95% was the highest stable RH value that could be maintained using the control device that was utilized herein and is close to the highest value (98%) that was utilized in an earlier study (Maus *et al.*, 2001) to simulate the highest possible humidity. The minimum storage period (8 hr) was the duration of a general one-day work shift, and the maximum (96 hr) was the period for which masks are worn in a week of work and equivalent to five days that was utilized in another study (Maus *et al.*, 2001).

Effects of Loaded Bacteria on Filter Performance

Two important indicators of filter performance were evaluated after airborne bacteria were loaded, including breathing resistance (BR) through the test filter and collection efficiency (CE). To measure BR, the vacuum pump was set to five flow rates, ranging from 2 to 10 L min^{-1}, corresponding

to light to extremely heavy workloads and including 6.28 L min^{-1} (face velocity of 8.3 cm s^{-1}). The pressure drop (Δp, ”H$_2$O) through the filter was monitored using an inclined manometer (series 200, Dwyer, USA). The Δp of a filter that was loaded with BS spores for 30 min was compared to that of an unloaded filter.

The CE and penetration rate (P) of the test filter were measured using a scanning mobility particle sizer (SMPS; TSI 3034, St. Paul, MN, USA), which measured the real-time concentration (# cc^{-1}) of airborne potassium sodium tartrate tetrahydrate (PST) particles with sizes from 20.2 to 594 nm, covering the most penetrating size, to investigate the efficiency of physical collection of the test mask. The filter quality factor (q_f) combines P and Δp, and is an index of filter media performance (Huang *et al.*, 2013). The term q_f is defined as follows.

$$q_f = \frac{ln(1/P)}{\Delta p} \tag{2}$$

Both P and Δp should be considered in ranking respirators. Based on the definition of q_f, a lower P and Δp yield a higher q_f.

Statistical Analysis

Experimental data were analyzed using conventional statistical tools in SPSS software, version 17.0 (SPSS Inc., Chicago, IL). Analysis of variance with *post hoc* Tukey's HSD multiple comparisons was used to compare the performances of four elution methods, the effects of storage temperature and relative humidity on RS, and the effects of bacterial loading on the collection efficiencies of the masks (McCullough *et al.*, 1997). After linear regression between the Δp and flow rate was carried out, analysis of covariance was used to test the assumption of homogeneity of the regression slopes to confirm the effects of bacterial loading. The statistics were considered significant at *p* < 0.05.

RESULTS AND DISCUSSION

Optimum Volume of Nutrients for Spiking into Bacteria-Laden Filters

When AS was used as a source of nutrients, overflow occurred at 1.7 mL for N95 and 0.7 mL surgical masks (Fig. 3). The RS of BS spores that were eluted from N95 increased from 11.6% to 39.5% as the volume of AS increased from 0.5 to 1.5 mL (Fig. 3(a)). When 1.5 mL of AS was dropped onto the N95 filter, the surface tension of the liquid caused it form a sphere-like droplet that was attached to the hydrophobic fiber matrix and then absorbed slowly into the filter matrix. When 3 mL of AS was added, once overflow occurred, RS declined to as low as 7.0%.

For the surgical masks, RS increased from 35% to 148% as the volume of AS increased from 0.1 to 0.5 mL before overflow occurred (Fig. 3(b)), and remained in the range of 40% to 70% after overflow. To increase RS, 1.5 and 0.5 mL of AS were used in the remaining trials of N95 and surgical masks, and capacities of liquid were calculated to

(a) N95 FFR
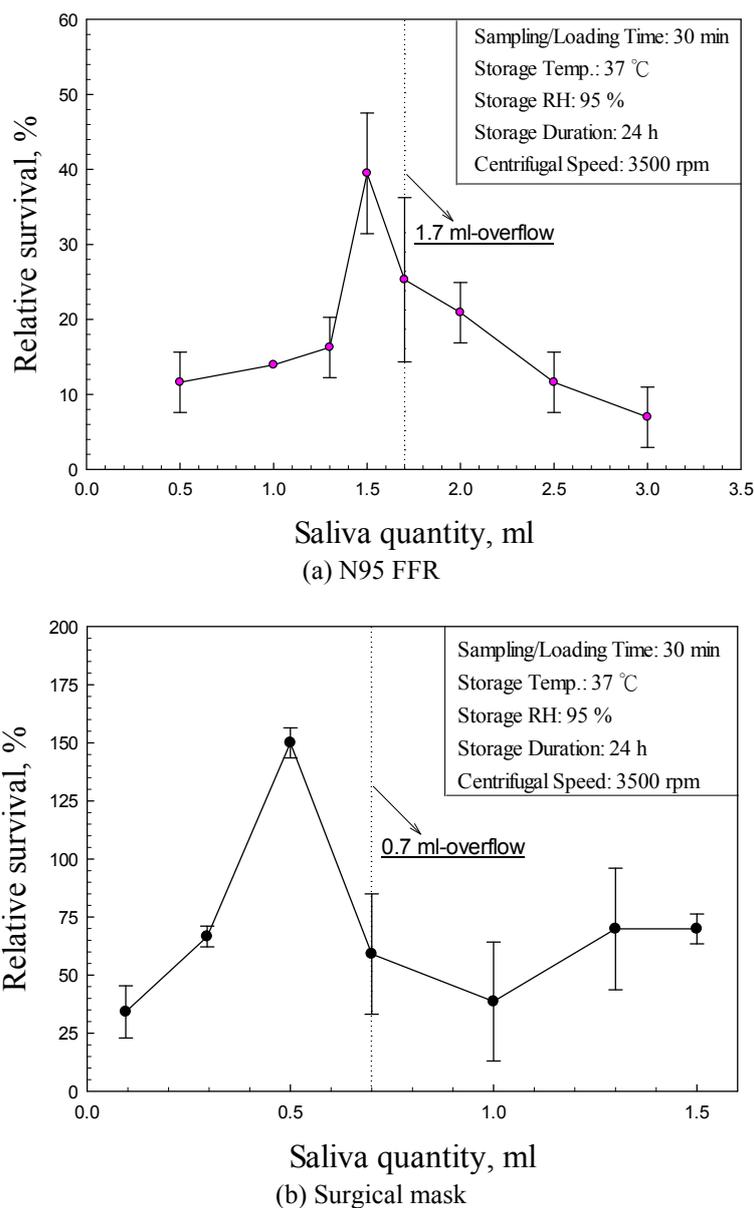
(b) Surgical mask

Fig. 3. Relative survival of *B. subtilis* spores loaded with various volumes of artificial saliva on (a) N95 FFR and (b) surgical mask.

be 0.120 and 0.056 mL cm^{-2}, respectively. Roberge *et al.* (2012) found the mass of the test FFRs increased by 0.15–0.28 g during 1–2 hr of usage owing to water vapor retention. Since medical staff in Taiwan sometimes use masks for more than 8 hr, as many did during the SARS outbreak, the masks may collect over 1.5 mL of liquid. In practice, if users sweat into or spill liquid on the mask in excess of its capacity, the liquid will run off it. The maximum volume that was determined in the AS tests was utilized in the AP tests.

The liquid capacity of the filter is affected by its constituent materials, thickness and wick immersion effect. The filtration matrices of the test masks both comprise hydrophobic nonwoven PP. However, when liquid is added to the fiber to simulate human sweat or saliva that is secreted the mask is worn, the wick immersion effect of

the fiber causes the liquid to be retained between the fibers. In this study since the N95 mask was thicker than the surgical mask, the volume of nutrient that was added to the N95 mask greater than that to the surgical mask.

When AS was dropped onto the surface of the surgical mask, no sphere-like droplet formed. Rather, AS was absorbed into the inner filter, which it did at a faster rate than for the N95 mask. In this case, the surgical mask became saturated with 0.7 mL of AS (Fig. 3(b)).

A positive correlation was identified between bacterial survival and the volume of liquid that was added to either mask. The results reveal that adding nutrients increased the survival of the loaded bacteria. For example, adding AS to the surgical mask yielded an RS of more than 100%, meaning that bacteria multiplied since the number of cultivable bacteria following storage exceeded that before.

Determination of Elution Method

The mean RS of the BS spores that were eluted using the centrifuge was observed to be 4.7% at a centrifugal speed of 1500 rpm (352 × g), increasing to 37.2% at 3500 rpm (1917 × g) (Fig. 4), suggesting that BS spores detached from the filter fiber. The highest mean average RS value was reached at 3500 rpm, and RS fell to 2.3% at 4500 rpm (3170 × g), indicating that the BS spores died as a result of the stronger centrifugal force. To remove biological particulates from the filter media, energy must be applied to overcome the forces by which the bioaerosols are attached to them (McCullough *et al.*, 1998). However, applied energy (over 3500 rpm) can reduce the cultivability of collected bacteria. Notably, these recovered colonies cannot provide the actual number of bacteria on the filter because some bacteria are likely to remain in the filter matrix, even when the most effective elution method is used (Wang *et al.*, 1999). To increase RS, the centrifuge speed was set to 3500 rpm in all subsequent trials.

Once the centrifuge speed was set, the mean RS values of the endospores that were collected by four elution methods were obtained and compared in Fig. 5. When the orbital shaker was used, the mean RS values of BS for filters without nutrition, using sterilized water and AS, were 0%, 4.4% and 15.3%, respectively. When the ultrasonic oscillator was used, RS values of 0%, 19.7% and 30.7% were obtained, while the vortex yielded RS values of 28.5%, 35.1% and 37.3%. The samples that were eluted using the centrifuge (at 3500 rpm for ten min, followed by vortexing for one min) had RS values of 32.9%, 37.3% and 41.6%, respectively. Since the centrifugal elution method yielded the highest RS values, it was utilized in all subsequent trials.

Relationships between RS of Bioaerosols and Both Nutrients and Storage Conditions

Fig. 6 displays the effects of storage conditions on the RS of BS on the N95 mask to which had been added AS. Figs. 6(a), 6(b) and 6(c) present the results for samples that had been stored at RH 95%, 60% and 40%, respectively. The three nutrient conditions were 1.5 mL of AS, 1.5 mL of sterilized water, and no nutrient. Owing to the overlapping error bars, the experimental points were shifted to compare easily the RS and its standard deviations under various storage conditions.

The RS values of the BS-N95-AS samples were all below 100%, regardless of RH, and the RS increased with the storage period from 8 hr to 24 hr, but decreased with the storage period from 24hr to 96 hr. The maximum RS (39.2%) was obtained at 95% RH and 37°C.

To compare the RS values of BS spores at various storage temperatures and RH levels, Fig. 6(d) plots pooled data concerning samples that were stored for 24 hr. When the samples were stored at 37°C, the highest (39.2%) RS was obtained at 95% RH, and the lowest (22.8%) RS was obtained at 40% RH. This result is consistent with an earlier study of the survival rates of BS spores at 37°C and an RH level of 85% (Wang *et al.*, 1999). The mean RS increased with RH. With respect to the effects of storage temperature and RH, the RS values of the samples at 37°C significantly exceeded those of those samples that were stored at 25°C ($p < 0.001$), and those of the 95% or 60% RH samples significantly exceeded those of the samples that were stored at 40% RH ($p < 0.001$). The interaction between the storage RH and temperature was statistically significant ($p = 0.003$). The worst-case conditions for BS spores seemed to be a high temperature (37°C) and high RH (95%), which represents a microenvironment that is similar to that of a worn mask. Under the worst condition, once the bacterial spores were loaded on the N95 mask, they could reproduce during the use or storage of the mask in under warm and humid conditions. Accordingly, RS increased from 8 to 24 hr, causing bacteria to survive at 96 hr.
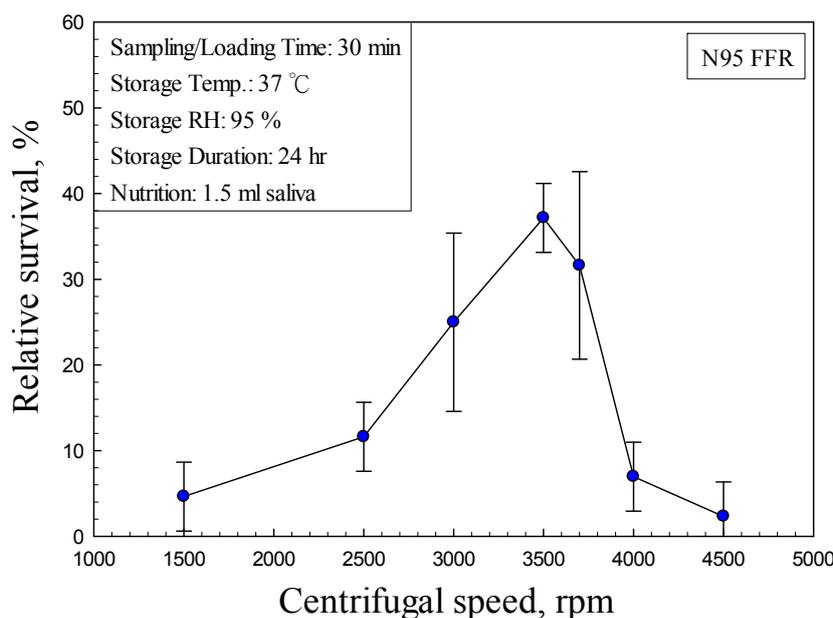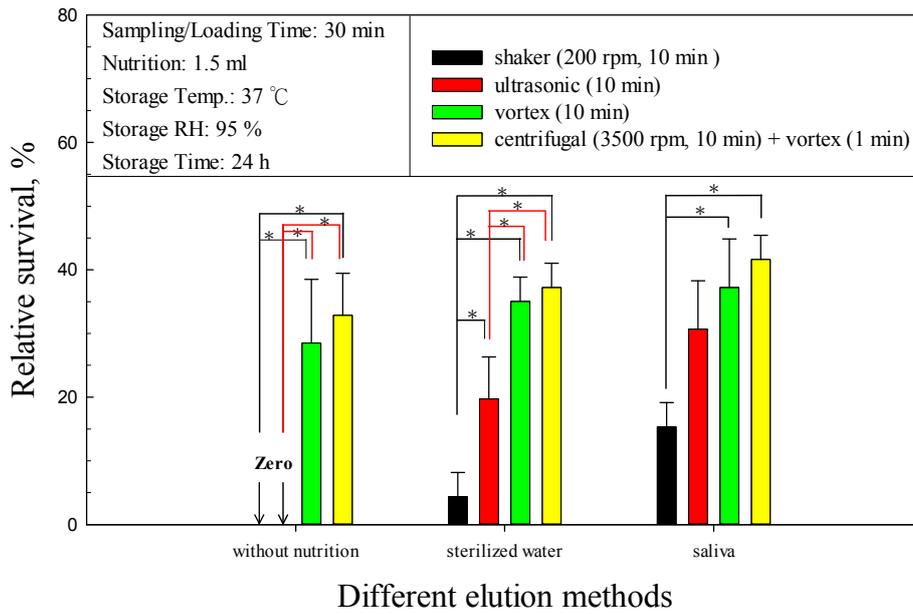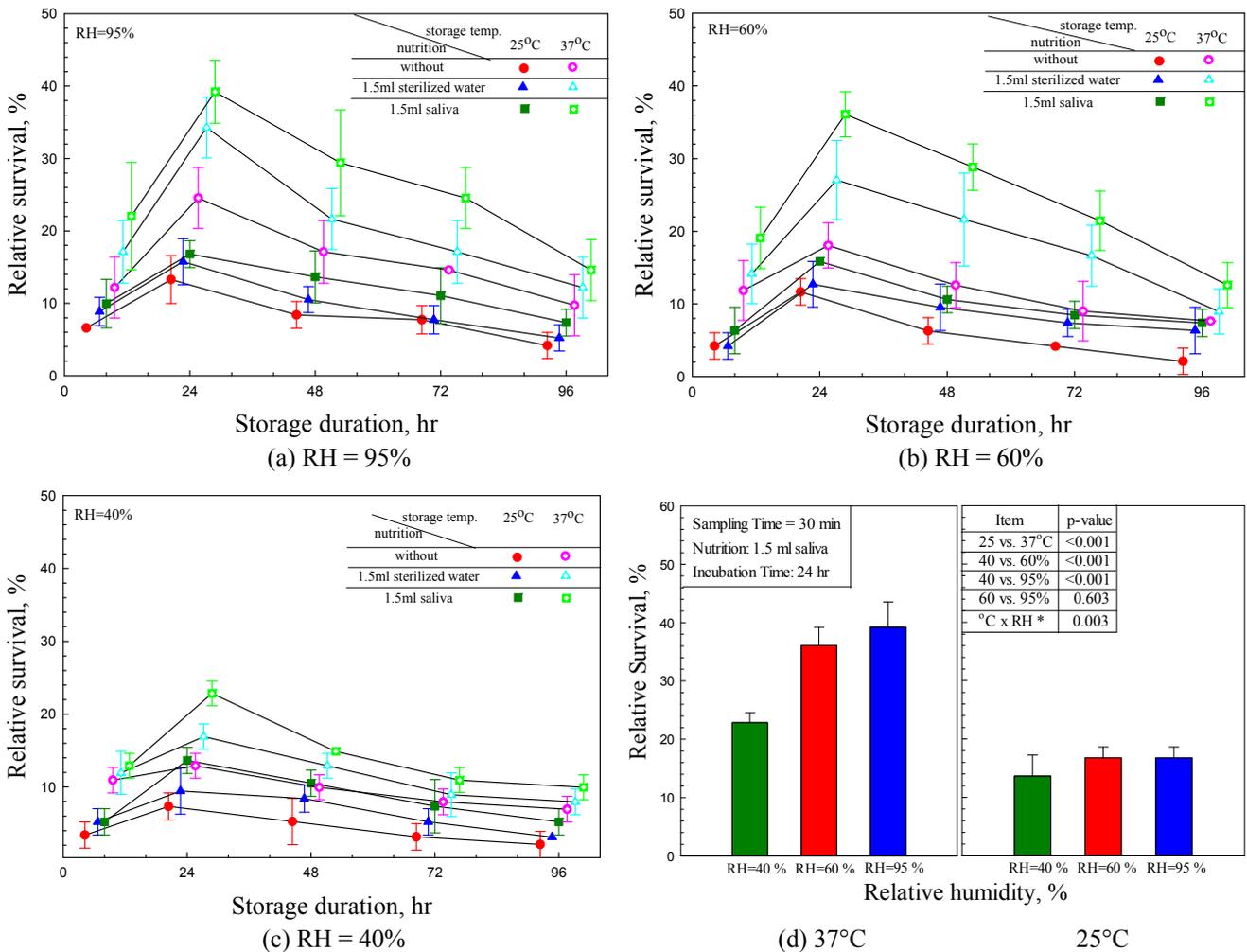

Fig. 4. Relative survival of *B. subtilis* spores loaded on N95 FFR and centrifuged at various speeds.

Fig. 5. Relative survival of *B. subtilis* spores loaded on N95 FFR, following elucidation by different methods; * indicates *p*-value < 0.05.

(a) RH = 95%

(b) RH = 60%

(c) RH = 40%

(d) 37°C   25°C

Fig. 6. Relative survival of *B. subtilis* spores loaded on N95 FFR after addition of artificial saliva and storage under various conditions: (a) RH 95%, (b) RH 60%, (c) RH 40%, from 8 to 96 hr and (d) for 24 hr.

The finding is consistent with the results of Wang *et al.* (1999). Viable bacteria pose a risk when they touch the surface of respirators if they are not carefully handled and stored. Hence, mask should be stored at a lower temperature (25°C or room temperature) if the mask is to be reused to ensure that as few bacteria as possible survive on the filters.

Since the worst storage condition was 37°C and 95% RH, the RS values of the N95 and surgical masks were compared under this condition (Figs. 7(a) and 7(b), respectively). When the challenge bioaerosol was EC, a few colonies recovered on most of the samples after storage and elution, and the maximum RS value was as low as 6.3%. The finding that the RS values of all of the EC samples were lower than those of the BS samples was similar to findings in other investigations

(Li *et al.*, 1999). Biological stress, including the generation of bioaerosols, impaction during sampling and dehydration after 30 min of sampling, reduced the RS of the environmentally sensitive EC, but not that of the robust BS endospores.

Also on N95 samples, the RS of BS increased to 133.2% and 317.0% after 8 h and 24 h of storage when AP was present as the nutrient. These were the worst-case values in this investigation, and higher than those obtained when AS was added (Fig. 7(a)). In contrast, the RS of BS on the surgical mask exceeded 100% after AS was added, and the lowest RS value was obtained when AP was added to the surgical mask (Fig. 7(b)). When AS was added to the test masks, the highest RS value of BS on the surgical masks (148.4%, as presented in Fig. 7(b)) was significantly higher

(a) N95 FFR
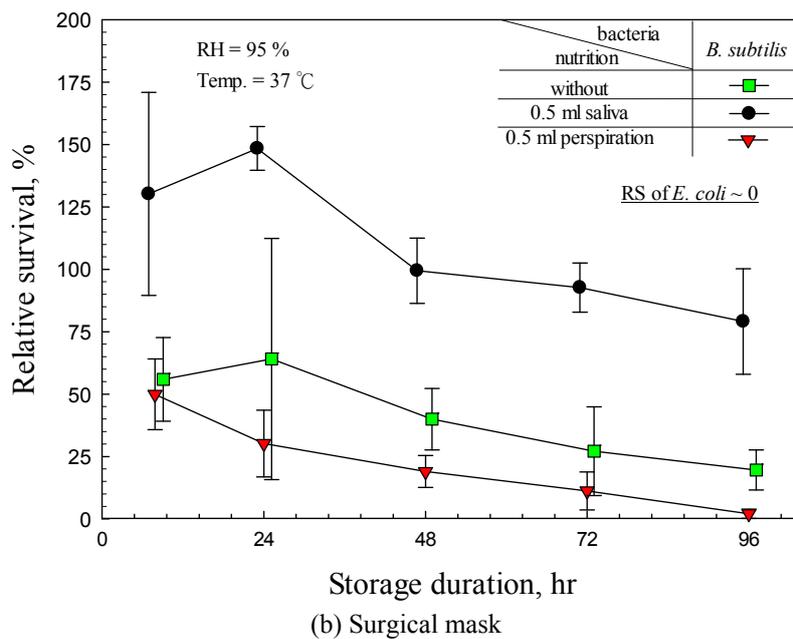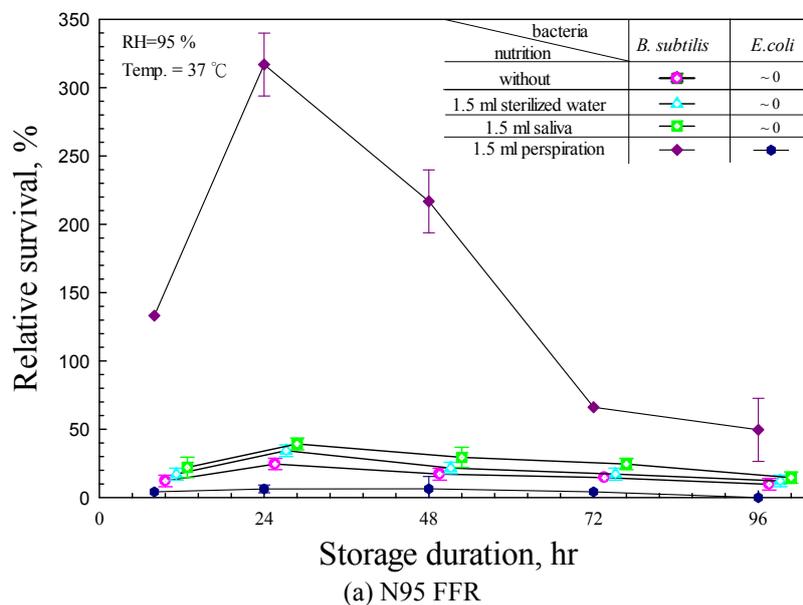
(b) Surgical mask

Fig. 7. Relative survival of *B. subtilis* and *E. coli* on (a) N95 FFR and (b) surgical mask with added nutrition and stored at 37°C and 95% RH (worst case).

than that on the N95 masks (39.5%, as shown in Fig. 7(a)) ($p < 0.001$), although the volume of AS that was added to the N95 FFR was three times of that added to the surgical mask. The rate of immersion of AS and the period of subsequent rehydration of the loaded bacteria contribute to this finding. Since the rate of immersion of AS was higher on the surgical mask, the dehydrated spores rehydrated more rapidly, allowing most of the loaded spores to survive and reproduce, yielding an RS of over 100%. For the N95 mask, the slower immersion of AS prevented most of the dehydrated spores that were trapped in the inner layer of the N95 filter from being rehydrated, causing their cultivability to be lost.

Nutrients affected the RS of bacteria. In the worst case BS-N95-AP samples, adding AP to the N95 FFR made available more of the AP nutrient, which was the applied nutrient available. The only other source of nutrients was the filter, itself. Since the pH value of the AP was 3.5, the strong acidity dissolved some unknown compounds from the fiber matrix of the N95 mask and effectively provided extra nutrients (trace elements) that promoted the multiplication of BS spores. However the fact that the surgical mask samples had the lowest values of RS revealed that not enough of trace elements were present in the fiber matrices, and so the low pH of the AP inhibited the survival of the loaded bacteria spores. Further study is required to analyze the trace contents of the mask materials. Users of masks would benefit from more information from manufacturers about the mask materials with respect to bacterial survival.

Effects of Loaded Bacteria on Filter Performance

For the N95 filters that were free of bacteria, Δp increased from 0.075 to 0.357 in-H_2O as the flow rate increased from 2 to 10 L min^{-1} (Fig. 8). When the N95 filter was loaded with BS spores for 30 min, $\Delta p$ linearly increased from 0.068 in-H_2O at 2 L min^{-1} to 0.313 in-H_2O at 10 L min^{-1}. However, no statistically significant difference was observed between the slope of the linear regression before and that after bacterial loading ($p = 0.233$). The measured values of Δp were less than 350 Pa, which is the upper limit that is specified in 42 CFR 84 subpart K (NIOSH, 2004).

The size of particles that most effectively penetrated (most penetrating size) the N95 mask was 49.6 to 51.4 nm before bacterial loading, which reduced the average CE value from 95.7% to 94.9% (Fig. 9(a)). The CE values of the masks that were loaded with BS spores were statistically significantly ($p < 0.001$) lower than those before loading, after adjustment were made for the size of particles a statistical model that incorporated q_f and particle diameter, the q_f of the filter was approximately 20 and significantly ($p < 0.001$) increased upon bacterial loading. Simulations by Huang *et al.* (2013) reveal that P and q_f both increased with increasing fiber diameter or decreasing packing density, consistent with the results herein. In this study the fiber diameter increased upon the attachment of BS spores, and adding liquid nutrients reduced the packing density. However, these findings warrant further investigation.

Limitations

A single elution method was utilized to compare the RS values obtained using various bacteria and masks. However, the selected elution method was ineffective for EC but yielded RS values under various storage conditions.

The AS and AP were sterilized before use to prevent bacterial contamination. Whether constituents of the AS and AP were destroyed upon sterilization or underwent the same reactions as those of the AS or AP could not be definitely determine, although most of the constituents were pure chemicals. In this investigation, AS and AP were autoclaved
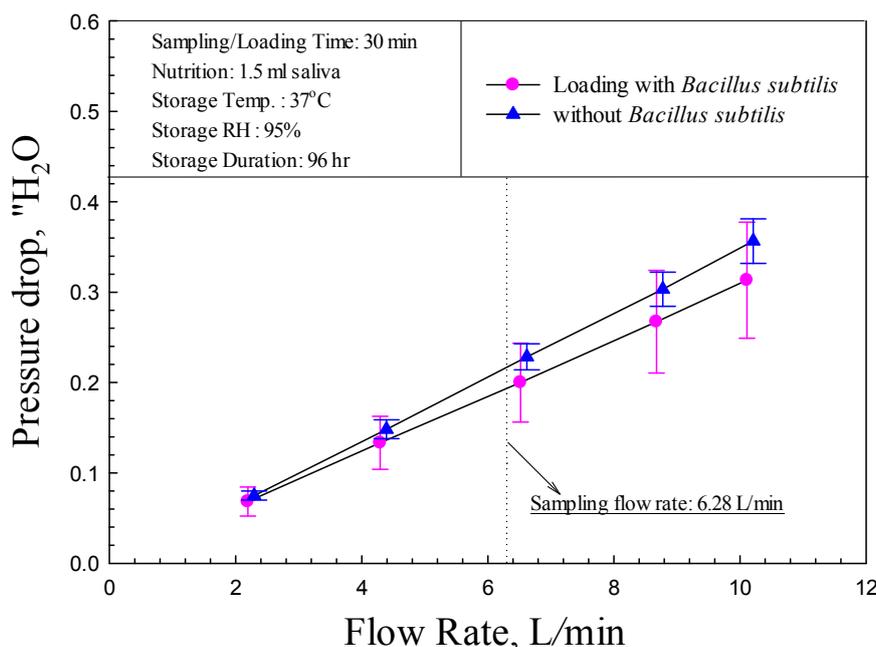
Fig. 8. Pressure drop through N95 FFR at various vacuum pump flow rates. No difference between the value of regression slopes ($p = 0.233$).
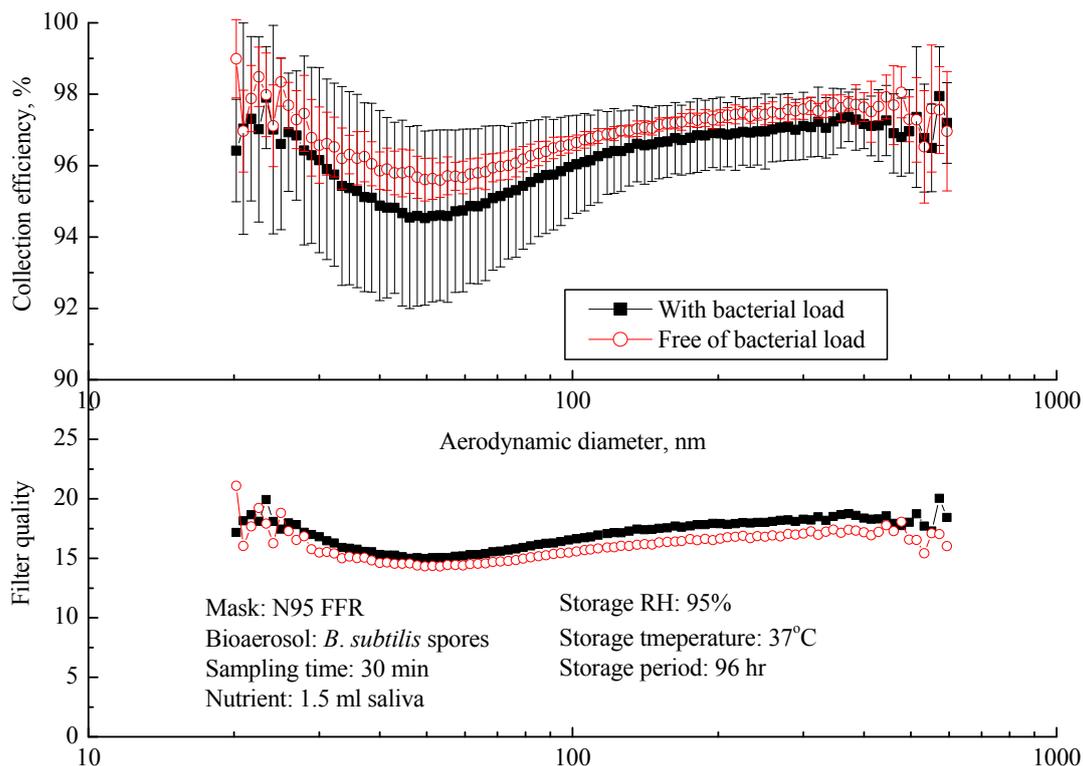
Fig. 9. Collection efficiency and filter quality factor of N95 FFR loaded with *B. subtilis*.

using a method that was described by Wang *et al.* (1999), but the results were based on sterilized nutrients because the artificial solution was sterilized before use.

Further research is required undertaken to determine the effects of filter materials, species of bacteria, nutrition, and re-entrainment of bacteria on the effectiveness of a given mask. For example, saliva and perspiration usually coexist on used masks. Since the effects of these two nutrients on the survival of bacteria were found to differ with the type of mask, the volume ratio of perspiration to saliva may affect bacterial survival in different ways for different masks, and this issue warrants further study in the future.

Healthcare organizations should use masks with fluid resistance protection which can be used in surgical settings, even though such fluid resistance masks (such as surgical N95 respirators) are not currently recommended by the Taiwan's health authority.

Based on the results of BS-N95-AS tests, the samples in the subsequent tests were stored at 37°C and 95% humidity, to represent the worst case. However, no one is likely to store their masks under such conditions - especially in developed countries where health care is typically provided in air-conditioned indoor environments. Alternatively, masks may be stored outside or in unconditioned indoor spaces, but environmental conditions are unlikely to average 37°C and 95% humidity over 8–96 hr periods. These facts represent a major limitation of this investigation and the "worst-case" conditions herein apply only when used masks are placed in a zipped bag or a pocket next to the human body.

Recently, the effect of a filter's characteristics on its performance was investigated by simulation (Huang *et al.*,

2013); the results thus obtained, such as the change in filter diameter and packing density upon bioaerosol loading and storage, require verification.

CONCLUSION

AS and AP can both promote the survival of BS spores; AP is especially effective in N95 FFRs and AS is especially effective in surgical masks under worst-case conditions. The pH value of these nutrients and the trace components in the mask have important roles in the process of bacterial reproduction, and this fact requires further investigation. Airborne EC cells exhibited poor survival in all tests. Respirators should be stored in a cool and dry location at, for example, room temperature and low humidity following any possible contamination with a bacterial pathogen, and this recommendation supports existing NIOSH guidelines for FFR reuse. Bacterial survival on surgical masks tends to exceed that on N95 FFRs. Therefore, surgical masks are strongly recommended to be discarded after one use. The bioaerosol load did not significantly influence the Δp of the N95 FFRs, but significantly increased penetration and q_f in this investigation. Additionally, since the optimal volumes of added liquid differed between the two kinds of test mask, the optimal volumes of added liquid for masks that are made of various materials should be assessed before similar research is performed in the future.

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